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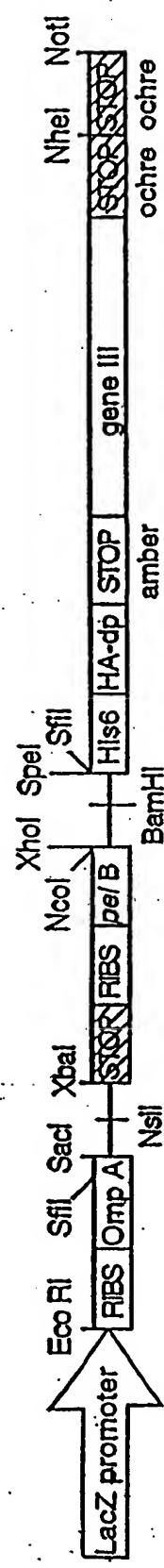


Fig. 1

Human Antibody sequence (TT sequence) (SEQ ID NO: 54)

Heavy Chain: cloning sites Xho I and Spe I are underlined

1 11
gag gtg cag ctg CTC GAG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG CTG AAG
glu val gln leu leu glu gln ser gly ala glu val lys lys pro gly ser ser val lys
21 31
GTC TCC TGC AGG GCT TCT GGA GGC ACC TTC AAC AAT TAT GCC ATC AGC TGG TGT CGA CAG
val ser cys arg ala ser gly gly thr phe asn asn tyr ala ile ser trp val arg gln
41 51
GCC CCT CGA CAA GGG CTT GAG TGG ATG GGA GGG ATC TTC CCT TTC CGT AAT ACA GCA AAG
ala pro gly gln gly leu glu trp met gly gly ile phe pro phe arg asn thr ala lys
61 71
TAC GCA CAA CAC TTC CAG GGC AGA GTC ACC ATT ACC GCG QAC GAA TCC ACG GGC ACA GCC
tyr ala gln his phe gln gly arg val thr ile thr ala asp glu ser thr gly thr ala
81 91
TAC ATG GAG CTG AGC AGC CTG AGA TCT GAG GAC ACG GCC ATA TAT TAT TGT GCG AGA GGG
tyr met glu leu ser ser leu arg ser glu asp thr ala ile tyr tyr cys ala arg gly
101 111
GAT ACG ATT TTT GGA GTG ACC ATG GGA TAC TAC GCT ATG GAC GTC TGG GGC CAA GGG ACC
asp thr ile phe gly val thr met gly tyr tyr ala met asp val trp gly gln gly thr
121 131
ACG GTC ACC GTC TCC GCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC
thr val thr val ser ala ala ser thr lys gly pro ser val phe pro leu ala pro ser
141 151
TCC AAG AGC ACC TCT GGG GGC ACA GCG GGC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC
ser lys ser thr ser gly gly thr ala ala leu gly cys leu val lys asp tyr phe pro
161 171
GAA CCG GTG ACG GTG TCG TCG AAC TCA GGC GCC CTC ACC AGC GGC GTG CAC ACC TTC CCC
glu pro val thr val ser trp asp ser gly ala leu thr ser gly val his thr phe pro
181 191
GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTC CCC TCC AGC
ala val leu gln ser ser gly leu tyr ser leu ser ser val val thr val pro ser ser
201 211
AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG
ser leu gly thr gln thr tyr ile cys asp val asp his lys pro ser asp thr lys val
221 231
GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA act aqt
asp lys lys val glu pro lys ser cys asp lys thr ser

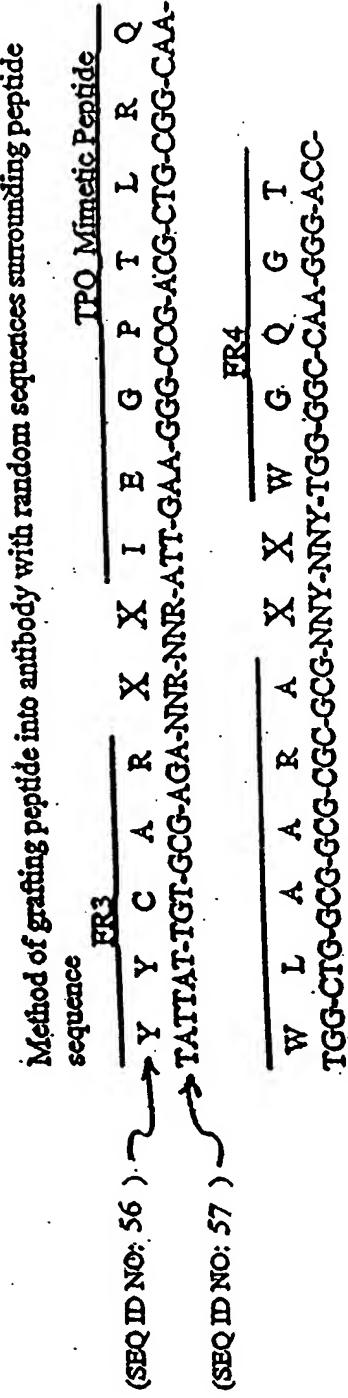
Fig. 2A

Human Antibody Sequence (TT sequence) (SEQ ID NO: 55)

Light Chain: cloning sites Sac I and Xba I are underlined

1 gaa ctc acg cag tct cca ggc acc ctg tct ttg tct cca ggg gaa aga gcc acc ctc tcc
glu leu thr gln ser pro gly thr leu ser leu ser pro gly glu arg ala thr leu ser 11
21
tgc agg gcc agt cac agt gtt agc agg gcc tac tta gcc tgg tac cag cag aaa cct ggc.
cys arg ala ser his ser val ser arg ala tyr leu ala trp tyr gln gln lys pro gly
41
cag gct ccc agg ctc ctc atc tat ggt aca tcc agc agg gcc act ggc atc cca gac agg
gln ala pro arg leu leu ile tyr gly thr ser ser arg ala thr gly ile pro asp arg 51
61
ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc aga ctg gag cct gaa
phe ser gly ser gly ser gly thr asp phe thr leu thr ile ser arg leu glu pro glu 71
81
gat ttt gca gtg tac tac tgt cag cag tat ggt ggc tca ccg tgg ttc ggc caa ggg acc
asp phe ala val tyr tyr cys gln gln tyr gly ser pro trp phe gly gln gly thr 91
101
AAG GTG GAA CTC AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT
lys val glu leu lys arg thr val ala ala pro ser val phe ile phe pro pro ser asp 111
121
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA
glu gln leu lys ser gly thr ala ser val val cys leu leu asn asn phe tyr pro arg 131
141
GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT
glu ala lys val gln trp lys val asp asn ala leu gln ser gly asn ser gln glu ser 151
161
GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC
val thr glu gln asp ser lys asp ser thr tyr ser leu ser ser thr leu thr leu ser 171
181
AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
lys ala asp tyr glu lys his lys val tyr ala cys glu val thr his gln gly leu ser 191
201
TTG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG Ttc tag a
leu pro val thr lys ser phe asn arg gly glu cys AMB 211

Fig. 2B



The TPO mimetic peptide was grafted into the heavy chain CDR3 region of the tetanus toxoid antibody. The peptide was flanked on either side by two random amino acids, shown as "X" in the figure.

Fig. 3

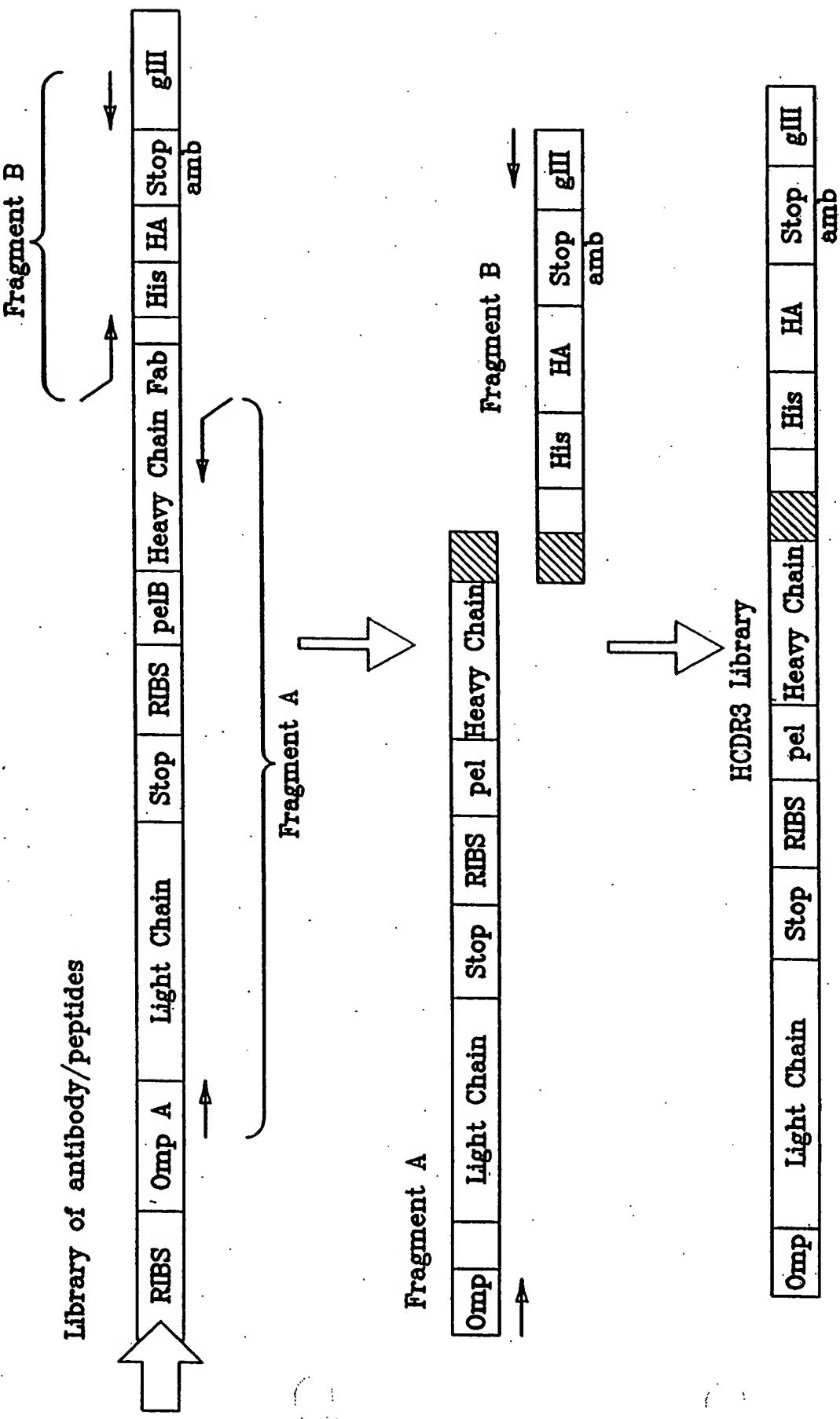


FIG. 4

AMINO ACID SEQUENCE

SEQ ID NO.

CLONE

CLONE	AMINO ACID SEQUENCE	SEQ ID NO.
X1a	Pro-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly- CCG-CCC-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCA-GGC	25 26
X1a-11	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly- GGG-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GGC-GGA	27 28
X1a-13	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly- GGC-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GGA-GGC	29 30
X1c	Trp-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val- TGG-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCC-GCT-GTC	31 32
X2a	Met-Ile-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Val-Gly- ATG-ATA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCC-GCT-GTC	33 34
X3a	Val-VAl-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val- TGG-GTA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCC-GCT-GTC	35 36
X3b	Gly-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp- GGG-CCG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCC-GAT	37 38
X4b	Leu-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val- TGG-CCA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCC-GCT-GTC	39 40
X4c	Ser-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val- TCA-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCC-ATC	41 42
X5a	Thr-Met-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val- ACA-ATG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCC-GAT	43 44
X5c	Thr-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val- ACG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCC-GCT-GTC	45 46
X7a	Thr-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Cys-Ser- ACA-CCG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCC-GCG-TGC-AGC	47 48
X7b	no peptide deletion mutant	
X7c	Gln-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp- CAG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCC-CAC	49 50

Fig. 5

pRL8

(SEQ ID NO: 60)

→ GGGAAATTGTAAGCGTTAATTTGTTAAAATTGCGTTAAATTGGTTAA.
ATCAGCTCATTTTAACCAATAGGCCGAAATCGCAAAATCCCTTATAAAATC
AAAAGAATAQACCGAGATAGGGTTGAGTGTGTTCCAGTTGGAACAAGAGT
CCACTATTAAAGAACCGTGGACTCCAACGTCAAAGGGCGAAAACCCTCTATC
AGGGCGATGGCCCACATACGTGAACCATCACCTAATCAAGTTTTGGGTGTC
GAGGTGCGTAAAGCACTAAATCGGAACCCCTAAAGGGAGCCCCCGATTTAGA
GCTTGACGGGAAAGCCGGCGAACGTGGGAGAAAAGGAAGGGAAAGAAAGC
GAAAGGAGCGGGCGTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGT
AACCAACACACCGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCAGGTGGC
ACTTTTGGGAAATGTGCGCGAACCCCTATTGTTTATTCTAAATA
TTCAAATATGTATCCGCTCATGAGACAATAACCCGATAAATGCTTCAATAAT
ATTGAAAAAGGAAGAGTATGAGTATTCAACATTCCGTGCGCCCTTATTCC
TTTTTGGGCATTGGCTTCCGTGTTTGTCAACCCAGAAAACGCTGGTGA
GTAAAAGATGCTGAAGATCAGTTGGGTGACGAGTGGGTTACATCGAACTGG
ATCTCAACAGCGGTAAAGATCCTGAGAGTTTCCGCCCCGAAAGAACGTTTCCA
ATGATGAGCACTTTAAAGTTCTGCTATGTGGCGCGTATTATCCCGTATTGA
CGCCGGGCAAGAGCAACTCGGTCGCCGATACACTATTCTCAGAAATGACTTG
GTTGAGTACTCACCAGTCACAGAAAAGCATCTACGGATGGCATACAGTAA
GAGAATTATGCACTGCTGCCATAACCATGAGTGATAACACTGCGGCCACTT
ACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTGACAAAC
ATGGGGGATCATGTAACTCGCCCTGATGTTGGGAAACCGGAAGCTGAATGAAG
CCATACCAAACGACGAGCTGTACACCAACGATGCTGTAGCAATGGCAACAAAC
GTTGCGCAAACATATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAAAT
TAATAGACTGGATGGAGGCGGATAAAAGTTGCAAGGACCACTCTGCGCTCGGC
CCTTCCGGCTGGCTGGTTATTGCTGATAAAATCTGGAGCCGGTGAGCGTGGGT
CTCGCGGTATCATTGCACTGGGCCAGATGTTAAGCCCTCCCGTATCGT
AGTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACA
GATCGCTGAGATAGGTGCTCACTGATTAAGCATTGGTAACTGTCAGACCAA
GTTTACTCATATATACTTTAGATTGATTAAAACCTCATTTTAATTAAAAGG
ATCTAGGTGAAGATCTTCTTGTATAATCTCATGACCAAAATCCCTAACGTGA
GTTTCTGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTCTT
GAGATCCTTTCTGCGCGTAATCTGCTGTTGCAAACAAAAACCGACCG
CTACCAAGCGGTGGTTGTTGCCGATCAAGAGCTACCAACTCTTCCGAA
GGTAACTGGCTTCAGCAGAGCGCAGATACCAAAACTGTCCCTCTAGTGTAG
CCGTAGTTAGGCCACCACTTCAGAAGACTCTGTAACGCCCTACATACCTCGC
TCTGCTAATCCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTGTGCTTA
CCGGGTGGACTCAAGACGATAGTTACCGATAAGGCCAGCGGGTGGCTG
AACGGGGGGTTGTCGACACAGCCAGCTGGAGCGAACGACCTACACCGA

Fig. 6A

ACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCGAAGGG
AGAAAGGCGGACAGGTATCCGTAAGCGGCAGGGTCTGGAACAGGGAGAGCGC
ACGAGGGAGCTTCAAGGGGAAACGCCTGGTATCTTATAGTCTCTGTCGGT
TTCGCCACCTCTGACTTGAGCGTCGATTTGATGCTCGTCAGGGGGCGG
AGCCTATGGAAAAACGCCAGCAACGCCCTTTACGGTCTGGCCTTTG
CTGGCTTTGCTCACATGTTCTTCTGCGTTATCCCCTGATTCTGTGGATAA
CCGTATTACGCCCTTGAGTGAGCTGAGCGAGGAAGCGGAAGAGCGCCAAACGACC
GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCAAACGCAA
CCGCCTCTCCCCGCGCGTGGCCGATTCAATTATGAGCTGGCACGACAGGTT
TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTATGTGAGTTAGCTC
ACTCATTAGGCACCCAGGCTTACACTTATGCTTCCGGCTCGTATGTTGTG
TGGAATTGTGAGCGGATAACAATTGAATTCAAGGAGGAATTAAAATGAAAAA
GACAGCTATCGCGATTGCACTGGCACTGGCTGGTCTCGTACCGTGGCCAG
GCGGCCGAGCTCGGCCATGGCTGGTGGCGAGCAGTAATAACAATCCAGCG
GCTGCCGTAGGCAATAGGTATTCATTATGACTGTCCTGGCACTAGCTA
GTTAGAATTGTAATCATGGTCAGCTGTTCTGTGTGAAATTGTTATOC
GCTCACAAATTCCACACAACATACGAGCCGGAAGCATAAAAGTGTAAAGCTGG
GGTGCCTAATGAGTGAGCTAACACATTAATGCGTTCGCTCACTGCCGC
TTTCCAGTCGGAAACCTGTCGTGTTACTAATGATGGTGTGGTAGGGCTAG
TTTGTCAACAAGATTGGCTCAACTTCTGTCACCTTGGTGTGGCTGGCT
TGTGATTACGTTGCAAGATGTAGGTCTGGTGGCCAAAGCTGCTGGAGGGCAC
GGTCACCACGCTGCTGAgGGAGTAGAGTCCTGAGGACTGTAGGACAGCCGGG
AAGGTGTGCA CGCCGCTGGTCAGGCCCTGAgGGCCCTGAgTTCCACGACACGTCGCCGG
TTCgGGGAAGTAGTCCTGACCAAGGCAAGCCCAGGGCCGTGTGCCCCAGAG
GTGCTTGGAGGAGGGTGCAGGGGAAGACCGATGGCCCTTGGTGGAG
GCTGCGGAGACGGTACCGTGTTACAGCAGAAACCTGGCCAGGCTCCAG
GCTCCTCATCTATGGTACATCCAGCAGGGCACTGGCATCCCAGACAGGTT
AGTGGCAGTGGCTGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC
CTGAAGATTGCACTGACTACTGTCAGCAGTATGGTGGCTACCGTGGTTC
GGCCAAGGGACCAAGGTGGAACCTAAACGAACTGTGGCTGCACCATCTGTCT
TCATCTTCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCCTGTGTTG
TGCCTGCTGAATAACTCTATCCCAGAGAGGGCAAAGTACAGTGGAAAGGTGG
ATAACGCCCTCCAATGGTAACCTCCAGGAGAGTGTACAGAGCAGGACAG
CAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGA
CTACGAGAAACACAAAGTCTACGCCCTGCGAAGTCaccatcaggcgttagtgcggcgtcac
aaagagcgtcaacggaggaggttaatTCTAGATAATTAGGAGGAATTAAAATGAA
ATACCTATTGCTTACGGCAGCCGCTGGATTGTTATTACTGCTGCCAACAG
CCATGGCCGAGGTGCAGCTGCTGAGATGAGCGATAAAATTATTCACCTGAC
TGACGACAGTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCTCGTC
GATTCTGGCAGAGTGGTGGTCCGTGCAAAATGATCGCCCCGATTCTGG
ATGAAATCGCTGACGAATATCAGGGCAAAGTACCGTTGCAAAACTGAACAT
CGATCAAAACCCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACT
CTGCTGCTGTTCAAAAACGGTGAAGTGGCGGCAACCAAAGTGGGTGCACTTG
TCTAAAGGTCA GTTGAAGAGAGTCTCGACGCTAACCTGGCGTACCCGTACG
ACGTTCCGGACTACGGTCTACTAGTccgaaaccgtcacccacgggcttccigcggtggccgc
atcgcccgltcggaggaaaaaglgaaaaacccgtaaagctcagaactccgagctggcgtccactgccaacatgcgtcgcaac

Fig. 6B

aggtggcacagctgaaacagaaaatgtaccatggcggtgtgctagt **GGCCAGGCCGCCAGCACCAT**
CACCATCACCATGGCGCATACCGTACGACGTTCCGGA
CTACCGCTCTGAGGGAGG
AGGGTGGTGGCTCTGAGGGTGGCGGTCTGAGGGTGGCGGCTCTGAGGGAGG
CGGTTCCGGTGGCTCTGGTTCCGGTGATTTGATTATGAAAAGATGGCAA
ACGCTAATAAGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTC
TGACGCTAAAGGCAAACCTGATTCTGCGCTACTGATTACGGTGTGCTATCG
ATGGTTCAATTGGTGACGTTCCGGCTTGCTAATGGTAATGGTGCTACTGGT
GATTGCTGGCTCTAATTCCCAAATGGCTCAAGTCGGTGACGGTGATAATTG
ACCTTAATGAATAATTCCGTCAATATTACCTTCCCTCCCTCAATCGGTTGA
ATGTCGCCCTTGTCTTAGCGCTGGTAAACCATATGAATTCTATTGATTG
TGACAAAATAAACTTATTCCGTGGTGTCTTGCGTTCTTTATATGTTGCCAC
CTTATGTATGTATTCTACGTTGCTAACATACTGCGTAATAAGGAGTCTA
AGCTAGCTAATTAAATTAAAGCGGCCGAGATCT

Fig. 6C

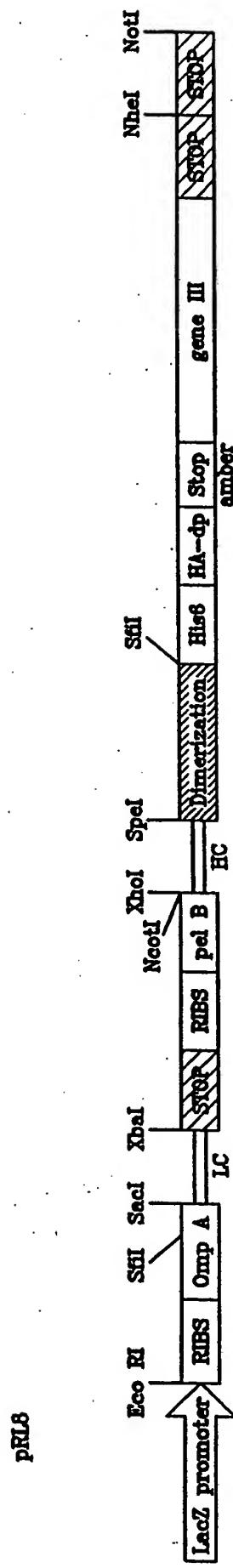


FIG. 7

(SEQ ID No: 52.)                                   <img alt="restriction site map for SphI" data-bbox="8495 800 8515 830

TPO Positive Clones nnk nnk nnk nnk
 1 2 IEGPTLROWLAARA 3 4

	Sample	nnk nnk		nnk nnk		Amino Acids		Amino Acids	
		1	2	3	4	1	2	3	4
HC CDR3	X1c	tgg	ctg	cct	gtc	Trp	Leu	Pro	Val
	X3a	gtg	gta	cct	gtt	Val	Val	Pro	Val
	X3b	ggg	ccg	ccc	gat	Gly	Pro	Pro	Asp
	X4b	ttg	cca	cct	gtt	Leu	Pro	Pro	Val
	X4c	tca	ctg	ccc	atc	Ser	Leu	Pro	Ile
	X5a	aca	atg	ccc	gtt	Thr	Met	Pro	Val
	X5c	acg	aca	cct	gtc	Trp	Leu	Pro	Val
	X7c	cag	aca	cct	eac	Gln	Thr	Pro	Asp
HC CDR2	24	ctt	tat	tct	aat	Leu	Tyr	Ser	Asn
	39	act	tac	ttt	cat	Thr	Tyr	Leu	His
	3	agg	atg	ctc	gag	Arg	Met	Leu	Glu
	7	aag	gaa	tct	aag	Lys	Glu	Ser	Lys
	8	gcg	cat	gtg	cag	Ala	His	Val	Gln
	10	cag	gag	att	agt	Gln	Glu	Ile	Ser
	11	cgg	aat	aat	ccg	Arg	Asn	Asn	Pro
	19	cag	cta	aat	tct	Gln	Leu	Asn	Ser
	25	agt	att	ttt	gtc	Ser	Ile	Phe	Val
	28	ggg	ccc	act	agt	Gly	Pro	Thr	Ser
LC CDR1	10	aag	ggt	gtt	agt	Lys	Gly	Val	Ser
	11	cat	ggg	gtg	gct	His	Gly	Val	Ala
	12a	cgt	acg	atg	gct	Arg	Thr	Met	Ala
	12b	cgt	ggt	gtt	aat	Arg	Gly	Val	Asn
	14	cgt	tcg	ctt	gcg	Arg	Ser	Leu	Ala
	16	cgg	ggt	gtt	gcg	Arg	Gly	Val	Ala
	18	agg	acg	gtg	tct	Arg	Thr	Val	Ser
	47	aag	ggg	gtg	gcg	Lys	Gly	Val	Ala
LC CDR2	1	aat	ccg	agg	ggt	Asn	Pro	Arg	Gly
	2	tcg	cct	ccg	agt	Ser	Pro	Arg	Ser
	3	tcg	cct	cgt	acg	Ser	Pro	Arg	Thr
	4	tcg	cct	tgg	cgt	Ser	Pro	Trp	Arg
	5	act	ccg	aat	tgg	Thr	Pro	Asn	Trp
	6	aat	cct	gcg	agg	Asn	Pro	Ala	Arg
	7	aat	ccg	tcg	ggg	Asn	Pro	Ser	Gly
	9	aat	cct	tat	tag	Asn	Pro	Tyr	Stop
	10	aat	ccg	ccg	tct	Asn	Pro	Arg	Ser
	11	aat	ccg	gat	gtg	Asn	Pro	Asp	Val
	12	tcg	ccg	tcg	ccg	Ser	Pro	Ser	Arg
	13	aat	cct	ctg	ttt	Asn	Pro	Leu	Phe
	14	aat	ctt	ggg	tat	Asn	Pro	Gly	Tyr
	15	aat	cct	att	agt	Asn	Pro	Ile	Ser
	16	aat	cct	cag	ccg	Asn	Pro	Gln	Arg
	18	aat	ccg	ccg	acg	Asn	Pro	Arg	Thr
	19	aat	ccg	cgt	ggg	Asn	Pro	Arg	Gly
	20	cgt	ttg	aga	ctg	His	Leu	Arg	Leu
	21	aag	tag	att	tat	Lys	Stop	Ile	Tyr
	23	aat	cct	ggt	aag	Asn	Pro	Gly	Lys
	24	aat	cct	cgt	ggg	Asn	Pro	Arg	Gly
	26	aat	cct	aat	gtg	Asn	Pro	Asn	Val
	27	tct	ccg	ccg	gtt	Ser	Pro	Arg	Val
	29	acg	cct	ccg	ggt	Thr	Pro	Arg	Gly
	30	ct	tag	tgg	tgg	Pro	Stop	Trp	Trp

Activity of Fab clones containing 2 TPO mimetic peptides

Fig. 10

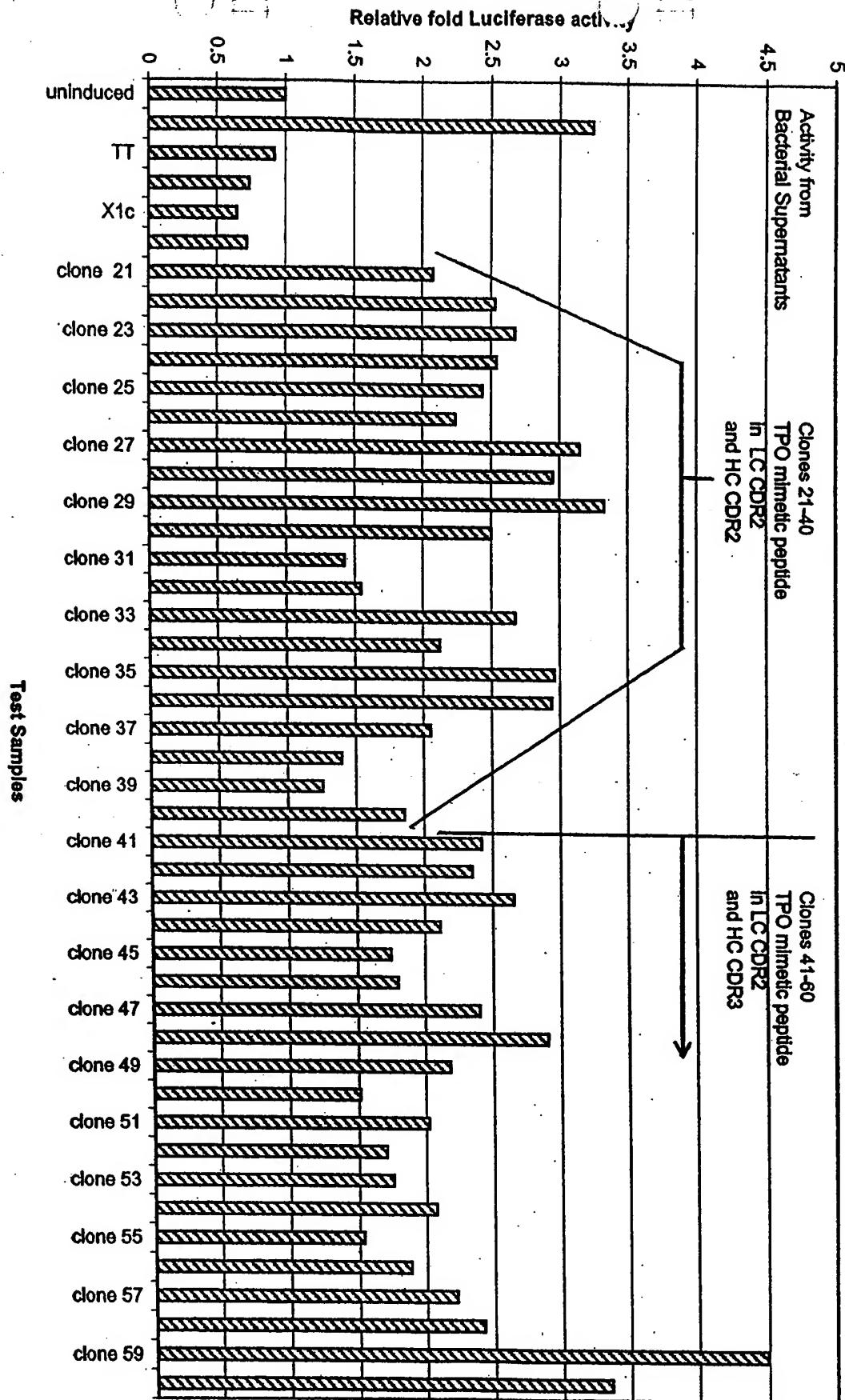


Fig. 11

Activity of Fab clones containing 2 or 3 TPO mimetic peptides

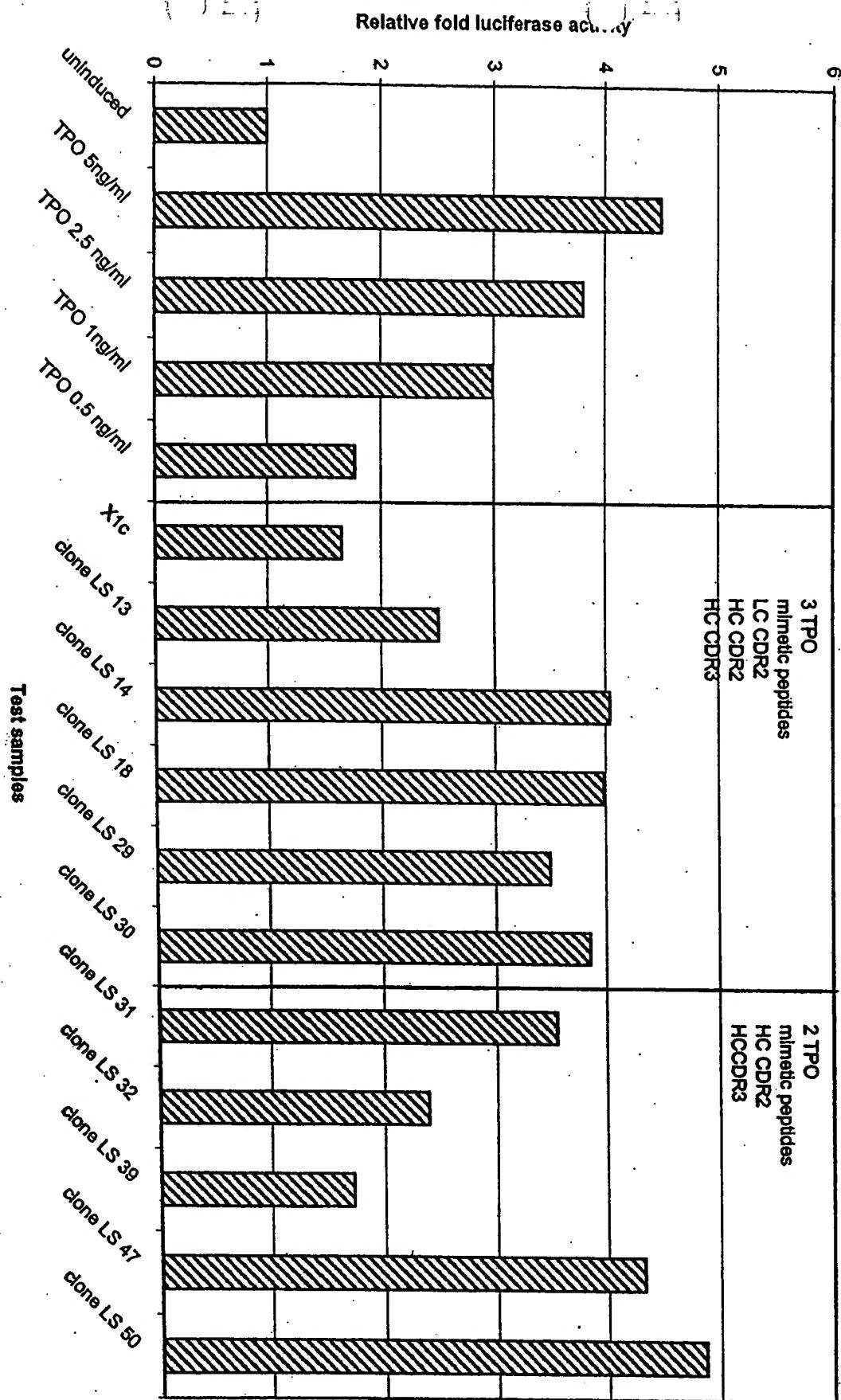
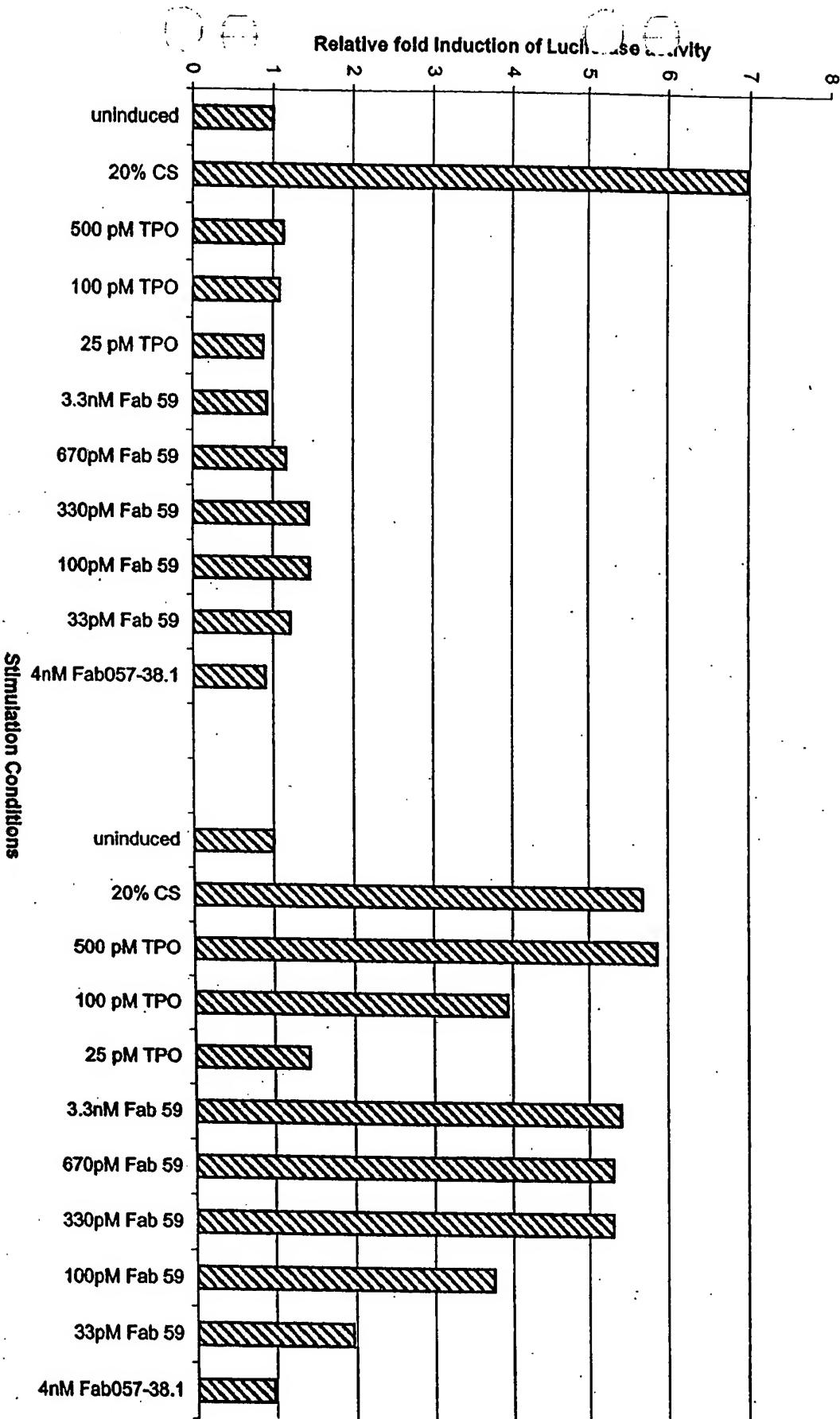


Fig. 12

TPO mimetic Activity of semi-purified Fab clone 59



(SEQ ID NO: 67)

SG1.1 - TPO Heavy Chain (Bold denotes TPO mimetic) Amino acid sequence:

MKIVSWVIL**F**LLSVTAGVHSQVQLVQSGAEVKPGASVKVSCKASGYIFSNYWIQW
VRQAPGQGLEWMGEILPGSGSTEY**T**ENFKDRVTMTRDTST**T**Y**M**ELSSLRSED
TAVYYCARLPIEGPTL**R**QWLAARAPVW**Q**GTLVTVSSASTKGPSV**P**LAPCSR
STSESTAALGCL**V**KDY**F**PEPVTVWSNSGALTSGVHTFP**A**VLQSSGLY**S**LSVVTV
PSSNFGT**Q**TYTC**N**VDHKPSNT**K**VD**K**VERKCCVE**C**PPCPAPPVAGPSV**F**LFPP**K**
KD**T**L**M**ISRT**P**EVTCVV**V**D**S**Q**E**DP**E**V**Q**FN**W**Y**D**G**E**V**H**NA**K**T**P**REE**Q**FN**S**TY
RVVSVLTVL**H**QDWLN**G**KEY**K**CKVSNKGLPSSIE**K**T**I**SKAK**G**Q**P**REP**Q**Y**T**L**P**PSQ
EEMTKNQV**S**LT**C**LV**K**GF**Y**PSDI**A**VE**W**ES**N**G**Q**PENNY**K**TPP**V**L**D**SD**G**SFF**L**Y**S**R
TVD**K**SRW**Q**EG**N**V**F**SCS**V**M**H**EA**L**H**N**Y**T**Q**K**SL**S**LG**K**

(SEQ ID NO: 68)

SG1.1 - TPO Heavy Chain (Bold denotes TPO mimetic) Nucleic acid sequence:

ATGAAGTGGAGCTGGGTATTCTCTCCTGTCA**G**TAACTGCCGGCGTCCA
CTCCCAGTCCAACTGGT**G**CAAT**C**GGCG**C**CG**A**GG**T**CAAG**A**AG**C**CA**G**GGGG**C**
TCAGTC**AA**AG**T**GT**C**CT**T**TA**AA**AG**C**TA**G**CG**G**CT**A**T**A**TT**T**TT**T**CT**A**TT**A**TT**T**GG**A**
TCA**A**TGGGT**G**CG**T**CAG**G**CCCC**GG****C**AGGG**C**CT**GG****A**AT**GG****A**TTGG**T**GG**G**AG**A**
TTAC**CC**GG**C**CT**T**GG**T**AG**C**ACC**G**A**AT**A**T**AC**CC****AA**AT**TT**AA**AG**ACC**G**T**GT**TA
CT**A**T**G**AC**G**CG**T**G**A**C**A**CT**T**CG**A**CT**A**GT**A**CT**A**GT**A**TC**A**AT**GG****A**GT**C**T**CC**AG**C**CT**G**
CG**A**T**CG**AG**GG****A**AC**GG****C**GT**T**AT**A**TT**G**CG**CG**GT**T**TC**CC**AA**T**GA**AG**
CC**G**AC**G**CT**G**GG**C**AA**T**GG**C**T**GG****C**GG**C**CG**C**GT**T**TC**CC**AA**T**GG**G**
GA**AC**CC**CT**GG**T**CA**T**GT**T**CG**A**GG**C**CC**T**CC**A**CC**A**AGGG**C**CC**A**TC**CG**GT**T**GG**C**CT**G**
CT**GG****G**CC**C**CT**G**CT**CC**AG**G**AG**C**AC**C**CT**CC**AG**G**AC**G**CC**G**CC**C**CT**GG****G**CT**G**
TGG**T**CA**AG****G**ACT**A**CT**T**CC**CC**GA**AC**GG**T**GA**C**GG**T**GT**C**GT**GG****A**CT**C**AG**G**CG**C**
C**C**T**G**AC**A**CG**G**GG**C**GT**G**CA**C**AC**C**CT**T**CC**GG****G**CT**G**TC**T**AC**A**GT**C**CT**C**AG**G**ACT**T**
A**C**T**C**CT**C**AG**C**AG**G**GT**T**GG**T**AC**C**CG**G**CT**C**CC**A**CT**T**CG**G**AC**C**CC**A**CC**A**
CT**A**CA**C**CT**G**CA**A**CG**T**AG**A**TC**A**CA**A**GG**C**CC**A**AC**A**CC**A**AG**G**T**GG****G**AC**A**AG**A**
A**G**TT**G**AG**C**GA**A**AT**G**T**T**GT**G**TC**G**AG**T**GT**CC**AC**C**CG**T**GG**C**AG**C**AC**A**CC**A**CT**G**T
G**C**AG**G**AC**G**GT**C**AG**T**CT**C**CT**T**CC**CC**AAA**A**CC**A**AG**G**AC**A**CC**C**TC**A**GT**G**
CT**CC**CG**G**AC**CC**CT**G**AG**G**T**C**AC**G**T**C**GT**G**GG**A**GT**G**AC**G**CC**C**AG**G**AA**G**
CC**C**CG**A**GG**T**CC**A**CT**G**GT**A**CT**G**GT**A**CG**T**GG**A**GT**G**AC**A**AT**G**CCA**A**AT**G**CC**A**
AG**A**CA**A**AG**C**CG**C**GG**G**AG**G**AC**G**CA**A**CG**T**GT**C**AC**A**CC**C**CT**G**CC**CC**AT**CC**AG**G**
G**G**AT**G**AC**A**AG**A**AC**C**AG**G**TC**G**AC**T**CC**C**GT**G**AC**A**GG**T**GT**A**CC**A**GG**C**CT**T**AC**A**
CC**A**CG**C**AC**A**TC**G**CC**G**GT**G**AG**G**AG**G**CA**A**AT**GG****G**AC**C**GG**G**AG**A**AC**A**
AC**A**AG**A**CC**A**CG**C**CT**CC**GT**G**GT**G**AC**T**CC**C**AC**G**GG**C**CT**C**CT**T**CC**C**TC**A**CG**C**
AG**G**CT**A**AC**C**GT**G**AC**A**AG**A**GC**A**GG**T**GG**C**AG**G**AG**GG****G**AA**T**GT**T**CT**T**CT**A**GT**G**
CC**G**T**G**AT**G**CA**T**GA**G**GG**C**T**C**TC**G**CA**C**AC**A**CC**A**CT**A**CA**C**AC**A**GA**A**AG**G**C**C**CT**T**CC**C**
GT**C**T**C**GG**G**AA**A**AT**G**

Fig. 13A

(SEQ ID NO: 69)

SG1.1 Light Chain Amino Acid Sequence

MDMRVPAQLLGLLLWLRGARCDIQMTQSPSSLSASVGDRVITTCGASENIYGALN
WYQQKPGKAPKLLIYGATNLADGVPSRFSGSQGTDFTLTISLQPEDFATYYCQ
NVLNTPLTFQGQTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC.

(SEQ ID NO: 70)

SG1.1 Light Chain Nucleic Acid Sequence

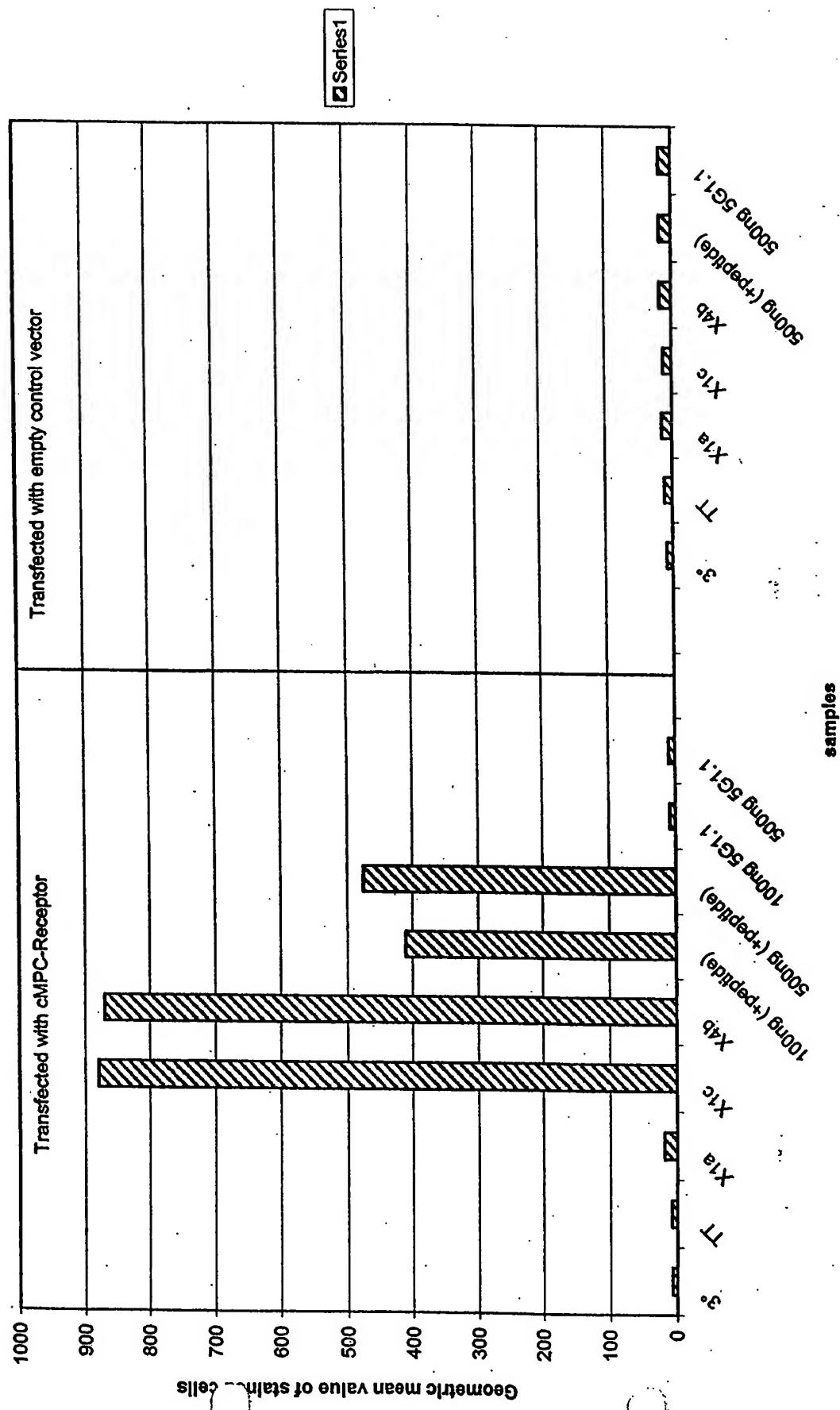
ATGGACATGAGGGTCCCCGCTCAGCTCTGGGGCTCTGCTACTCTGGCTCCG
AGGTGCCAGATGTGATATCCAGATGACCCAGTCCCCGCTCCCTGTCCGCCT
CTGTGGCGATAAGGGTCAACCATCACCTGCGCGCCAGCGAAAACATCTATGG
CGCGCTGAACCTGGTATCAACAGAAACCCGGAAAGCTCCGAAGCTTCTGATT
TACGGTGCACGAACCTGGCAGATGGAGTCCCTCTCGCTTCTGGATCCGG
CTCCGGAACGGATTCACTCTGACCATCAGCAGTCTGCAGCCTGAAGACTTC
GCTACGTATTACTGTCAGAACGTTAAATACTCCGTTGACTTTCGGACAGGG
TACCAAGGTGGAAATAAAACGAACCTGTGGCTGCACCATCTGCTTCATCTTCC
CGCCATCTGATGAGCAGTGAATCTGAACTGCCCTGTGTGCCCTGCTG
AATAACTTCTATCCCAGAGAGGGCAAAGTACAGTGGAGGGATAACGCC
TCCAATCGGGTAACCTCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGA
AACACAAAGTCTACGCCCTGCGAAGTCACCCATCAGGGCTGAGCTCGCCCGT
CACAAAGAGCTAACAGGGAGAGTGTAG

Note: Italics denotes leader sequence

Fig. 13B

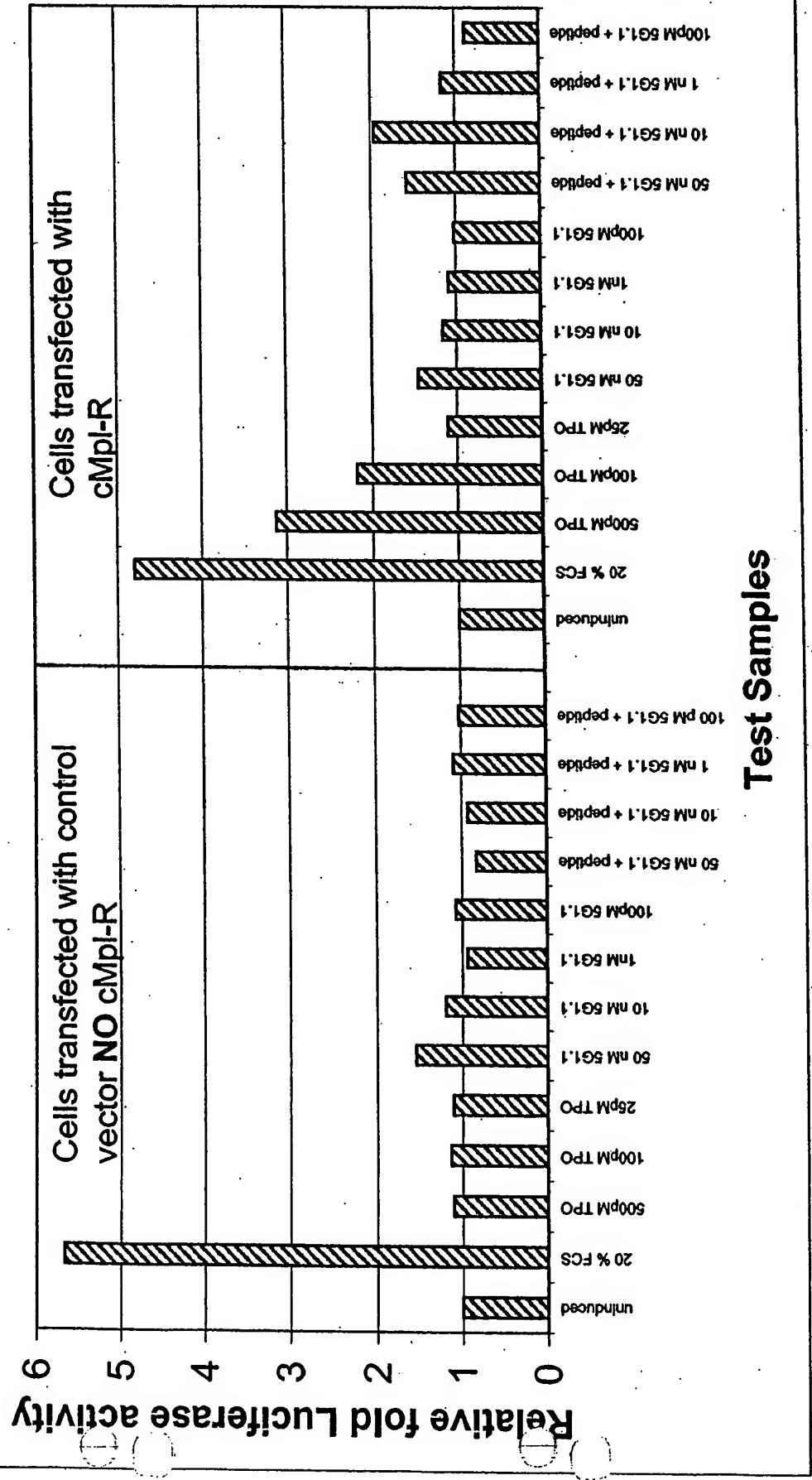
Fig. 14

FACS staining on transfected 293 cells



15

Activity of 5G1.1 containing the TPO mimetic Peptide



VARIABLE REGION OF 4-29 LIGHT CHAIN

(SEQ ID NO: 116)

GAC ATC CAG ATG ACC CAG TCT CCA TCC CTC CTC GCA TCT GAA GAC AGA GTC ACC ATC ACT TCC CGG AGT CAG	CDR1	29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 52a 52b 52c 52d 52e 52f 52g 52h 52i 52j
ATT AGT TGC CTC GCC TGG TAT CGG CGG AGA CCA CGG AGA GCA TAC CGT CCT TAA CTC CTC ATC TAT AAC CGG XAA GCT XAA	CDR2	ATT AGT TGC CTC GCC TGG TAT CGG CGG AGA CCA CGG AGA GCA TAC CGT CCT TAA CTC CTC ATC TAT AAC CGG XAA GCT XAA
AGC ATC CAG ATG ACC CAG TCT CCA TCC CTC CTC GCA TCT GAA GAC AGA GTC ACC ATC ACT TCC CGG AGT CAG	CDR1	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 27a 27b 27c 27d 27e 27f 28
ATT AGT TGC CTC GCC TGG TAT CGG CGG AGA CCA CGG AGA GCA TAC CGT CCT TAA CTC CTC ATC TAT AAC CGG XAA GCT XAA	CDR2	ATT AGT TGC CTC GCC TGG TAT CGG CGG AGA CCA CGG AGA GCA TAC CGT CCT TAA CTC CTC ATC TAT AAC CGG XAA GCT XAA

	CDTR3										
86 87 88	89 90 91	92 93	94 95	96 97	98 99	100 101	102 103	104 105	106 107		
mac mac	CAA CAG	TAT AAT	AGT TAC	CCT CCC	ACT TTC	GCG ACC	AAA AAA	GTG GTG	GAT GAT	ATC AAA	

Fig. 16

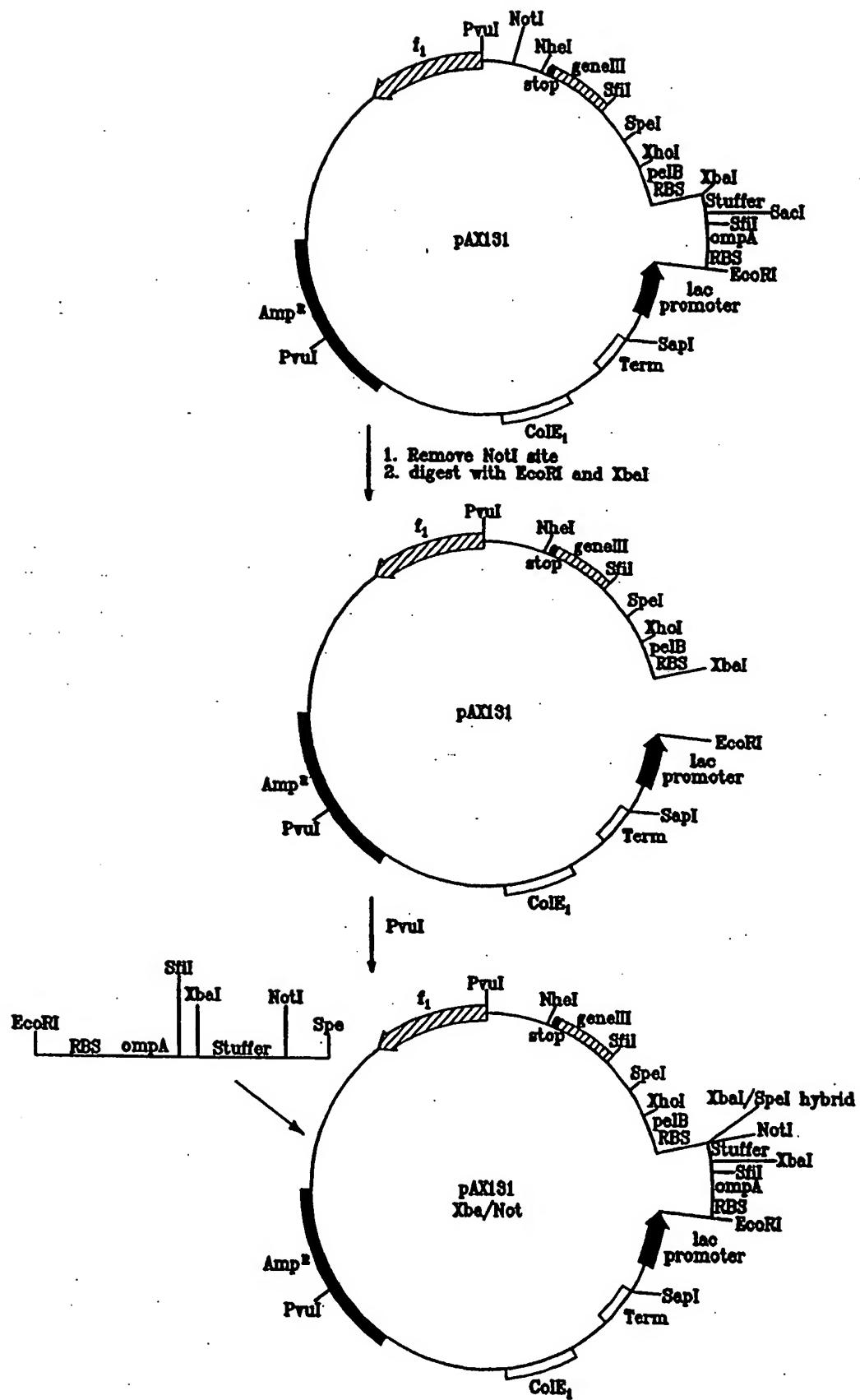
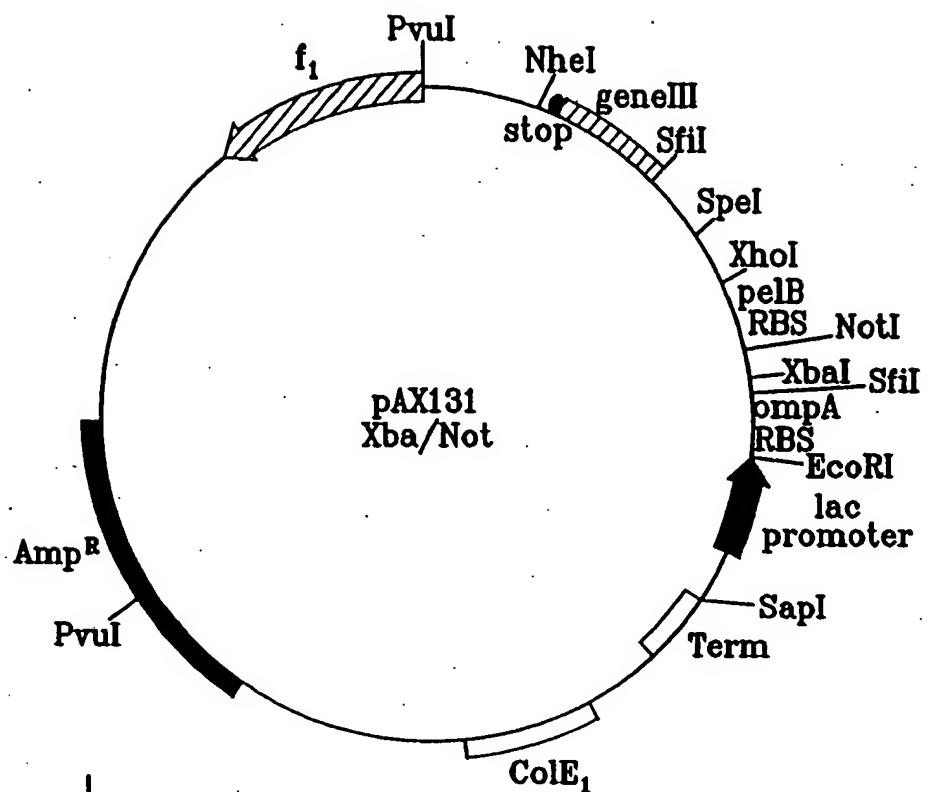


FIG. 17



Plus Kappa Constant Region

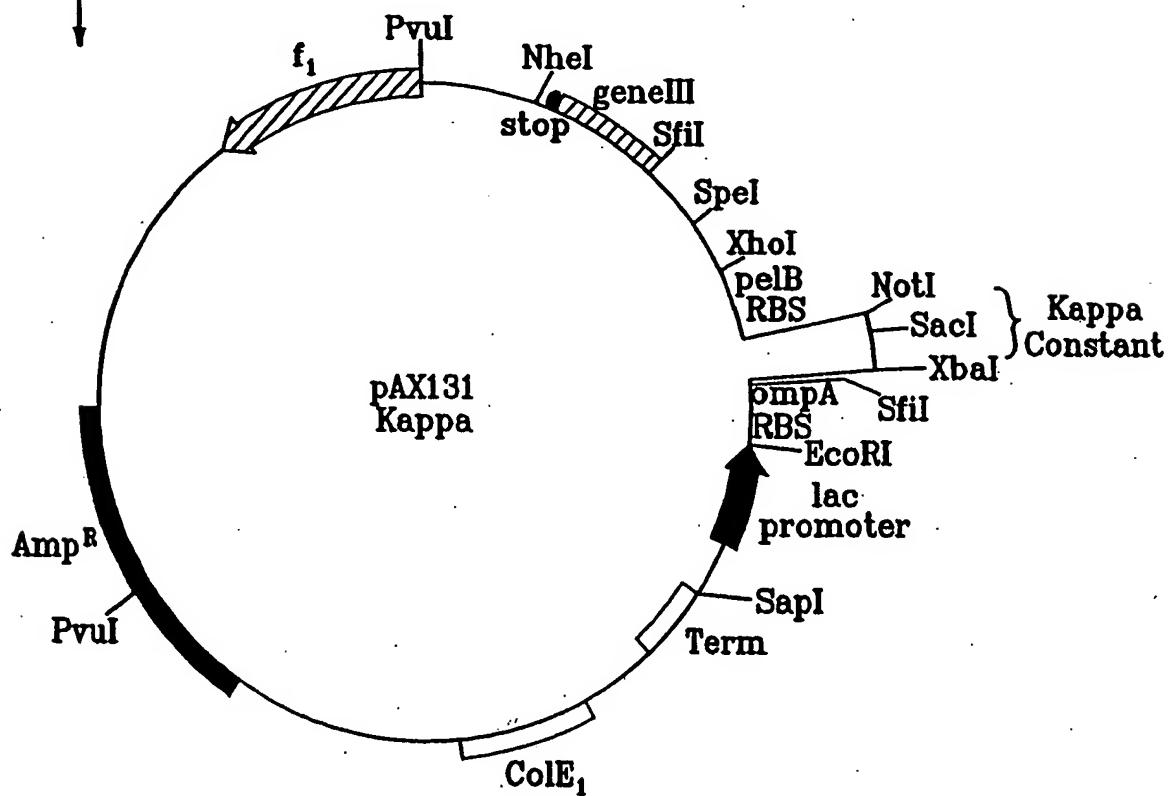


FIG. 18

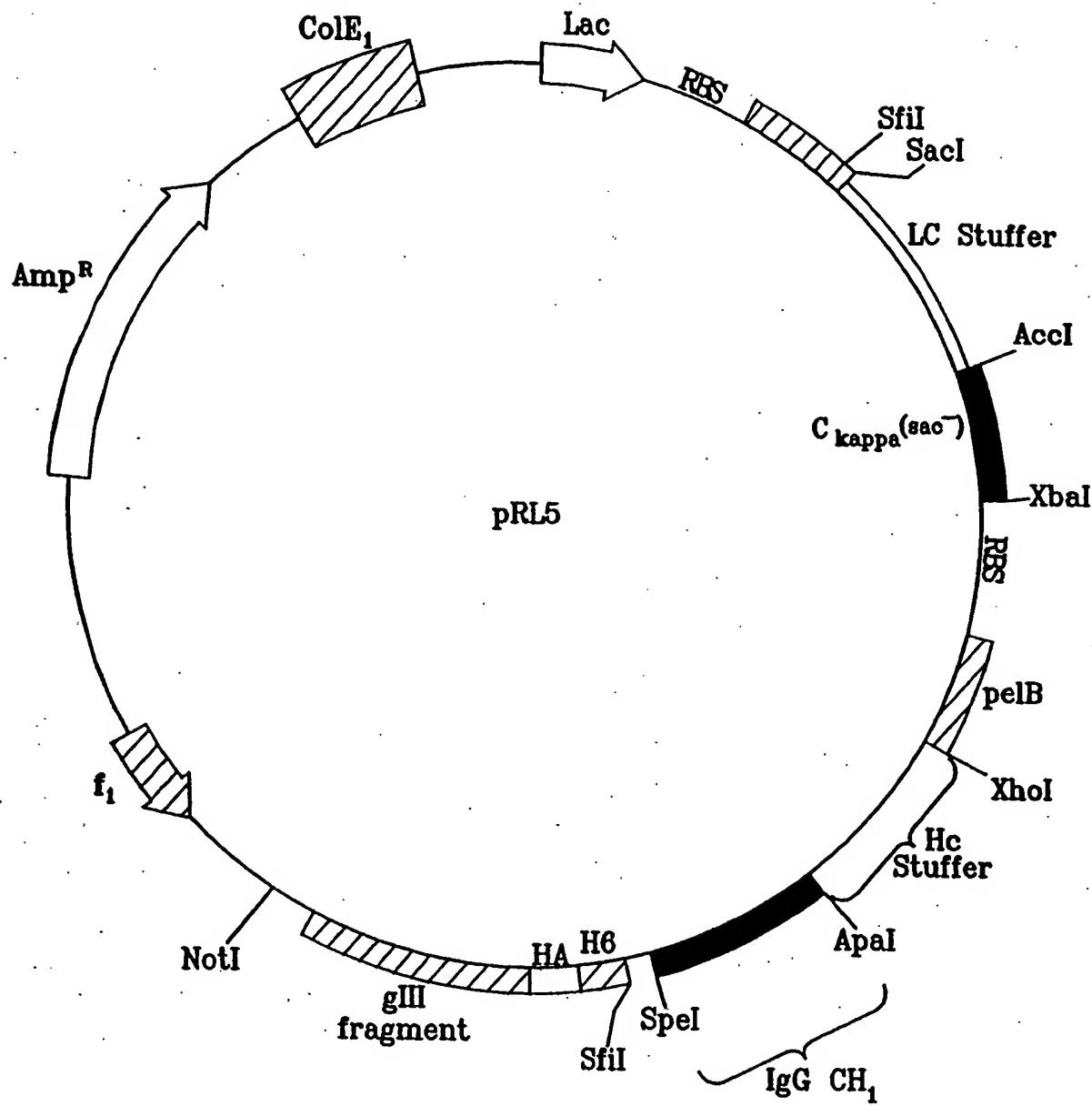
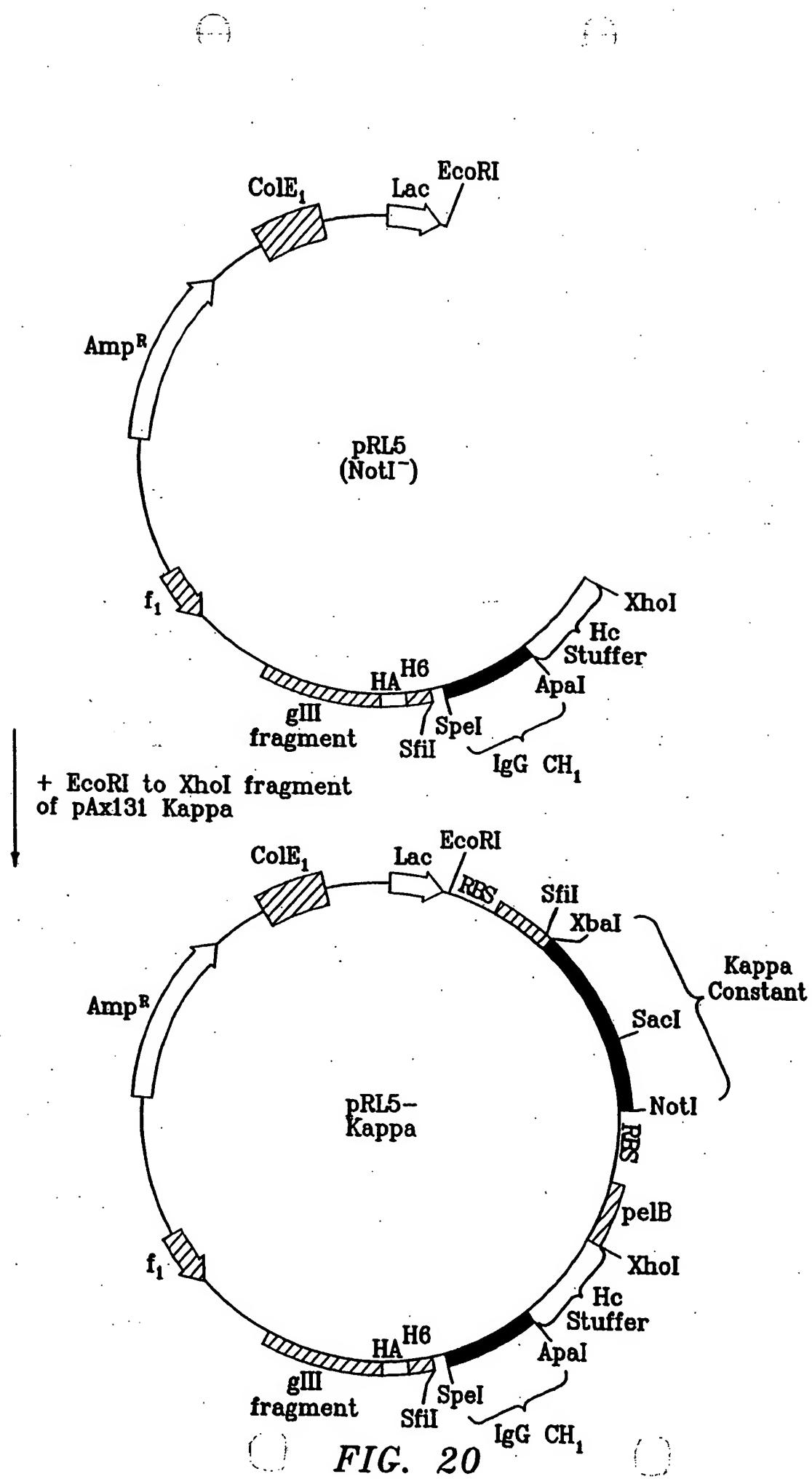


FIG. 19



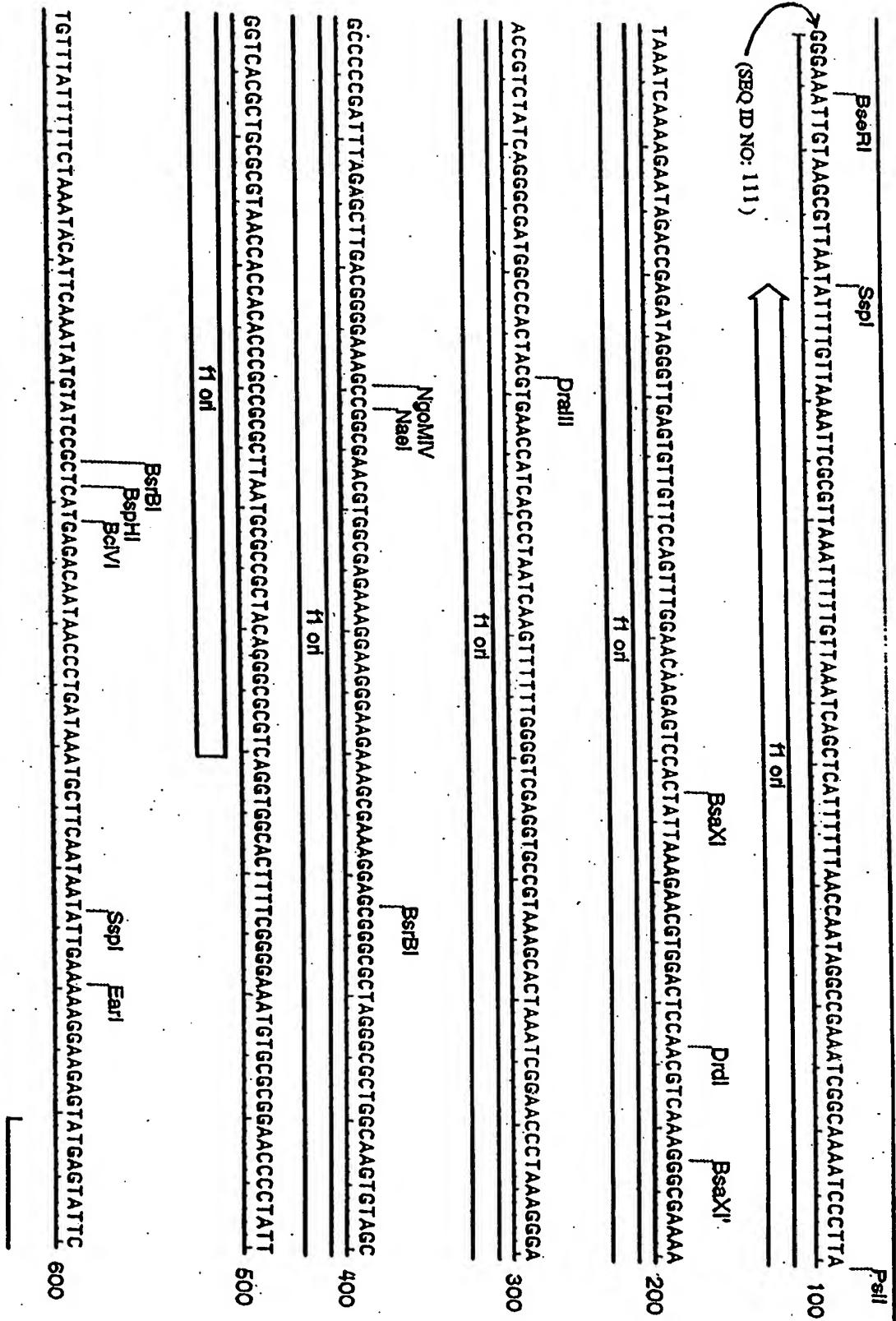


Fig. 21A

AACATTCCGTGTCGCCCTATTCCCTTTGGCCATTGGCCTCTGTTGCTACCCAGAACGCTGGTAAAGATGCTGAAGATCA 700

beta-lactamase

ApalI
BssH I
Eco57I

GTGGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGGGTAAGATCCTGAGAGTTGGCCCCGAAGAACGTTTCCAAATGATGAGCACTTTT 800

beta-lactamase

AclI
XbaI
PstI

AAAGTCTGCTATGTTGGGGTATTATCCCGTATGACGGGGCAAGAGCAACTCGGTGCCGATACTATTCAGAATGACTTGGTTGAGTACT 900

beta-lactamase

BglI

BglI

Scal

BstI

BstI

1000

CACCAAGTCACAGAAAGCATTTACGGATGGCATGGACAGTAAGAGAATTATGCACTGGCTGCCATAACCATGAGGTATAACACTGGGGCAACTTACTTCT 1000

beta-lactamase

PvuI

GACAACGGATCGGGAGGACCGAAGGGAGCTAACCGCTTTTGCACAAACATGGGGATCATGTAACTCGCCTGATCGTTGGAACCGGAGCTGAATGAAAC 1100

beta-lactamase

Fig. 21B

CCGTGGGGCTGGTGCCCCGGTGGCTGACGAGAGCGGGCCACGTAGTGTGCTGGCTCCGCCGCTGAGACACCCATGACGTCACATCCGCTACGA
3400

HC stuffer

GGGGCCACGTAGTGGCTCCGGCCTGAGACCCATGACGTCACATCC
plain

3400

Att! Eng!

BIBLIO

CCCTGGACGTCCTGGGGCGGCCACGGGCCAGGGAGCTACAGAGGTGGAGATCTCTGGAGGCCGCCACCGACTGTGCTGAGCAACCTGCGGGCTGGAGC

Volume 3

axial pressure (psi).

7

HC stuffer

Earl

GCTGTGGCTGTACCAAGAATGATGGCTGC

HC studie

100

HC stuffer

Fig. 21C

BsrGI BsrDI AccI FspI
 ATACCAACGACGGAGCTGTACACCACGATGCCTGTAGCAATGGCAACAGTTGCCAACTATTAACTGGCGAAACTACTCTAGCTTCCGGCAAC 1200

 beta-lactamase

Asel EclI
 AATTAATAGACTGGATGGAGGGGATAAAGTTGCAGGACCACTTCTGCCTCGGCTTCCGGCTGGCTGGTTATTCGCTGATAATCTGGAGCCGGGA 1300

 beta-lactamase

BpmI BsaI BstDI BmrI
 GCCTGGGTCTCGGGTATCATGCAGCACTGGGCCAGATGCTAAGCCCTCCGTATCGTAGTTACACGACGGGAGTCAGGCAACTATGGATGAA 1400

 beta-lactamase

AhdI
 CGAAATAGACAGATCGCTGAGATAGGTGCTCACTGATTAACCATGGTAACCTGTCAAGACCAAGTTACTCATATAACTTATGATTAAACTTC 1500

 beta-lactamase

Dral
 ATTTTTAATTAAAGGATCTAGGTGAGATCCTTTGATAATCTCATGACCAAAATCCCTAACGTTGACTTTGCTTCCACTGAGCGTCAGACCCGT 1600
 AGAAAAGATCAAAGGATCTCTGAGATCCCTTTCTGCCGTAACTGCTGCTTGCAACAAACACCGCTACCGACGGTGGTTGGTTGCCG 1700

 ColE 1 origin

Fig. 21D

Fig. 21E

Eco571

GATCAAGAGCTACCAACTTTTCCGAAGCTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCCTCTAATGTAGCCGTAGTTAGGCCACCACT 1800

colE I origin

AlwNI

TCAAGAACTCTGTAGCACCGCCTACATACCCGACTCTGCTTAATCCCTGTTACCACTGGCTGCTGCCAGTTGGCGATAGCTGCTTACCGGCTTGGACTC 1900

colE I origin

ApalI

AAGACGATAGTTACCGATAAGGCCAGCGGTGGCTAACGGGGTTCGTGCACACAGCCCAGTTGGAGCGAACGACCTACACCGAAGTGGAGATAC 2000

colE I origin

BssI

CTACACGGCTGAGCTATGAGAAAGGCCACCGCTCCCGAAGGGAGAACGGGGACAGGTATCCGGTAAGGGCAGGGTGGAACAGGAGAGGGCACCGAGG 2100

colE I origin

PstI

AGCTTCCAGGGGAACGGCCTGGTATCTTATAGCTGTGGGTTTGCACCTCTGACTTGAGCGCTGATTTGTGATGCTGTCAGGGGGCGGAG 2200

colE I origin

PstI

CCTATGGAAAAACGCCAGCAACGCCACCTTACGGTCTGGCTTGGCTTGGCTTACATGTTCTGCTTATCCCTGATTCTGTG 2300

colE I origin

GATAACCGTATTACCGCCTTGAGTGTACCGCTCGCCAGCGAACGACGGCGCAGCGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCCAA 2400

୪୭

Earl Sapl Bsa XI

TACCGAAACCGCCTCTCCCCGGCGTTGCCGATTCATAATGCCAGCTGGCACGAGTTCCGACTGGAAAGGGCAGTGAGGCCAACGCCATTAA
2500

Bsi
Asel

TGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTACACTTTAGCTTCCGGCTCGTATGTGTGGAAATTGAGCGGATAACAAATTGAATTCAAGG

TAACAAATTGAATT
JMBI ECON

Fig. 21F

AGTTAATTATGAAAAACCGCGATTGCGATTGGCTGGGGCTTAGATAACTGTGCTGCACCATCT 2700

B61

— Kappa constant —

leader —

۲۰۱

GTCTTCATCTTCCCCCATCTGGATGAGCAGTTGAATCTGGAACTGCCCTCTGTTGCTGCTGATAACTCTATCCCAGAGGCCAAAGTACAGT 2800

– Kappa constant

88

GGAAAGGTGGATAACGCCCTCCAAATCGGGTA
CTCCAGGAGACTGTCACAGAGCAGCAC
CTACAGCCAGCAGCACCTGACGCT
BbvCI Bpu10I BpiI 2900

- Kappa constant

ALWIN
BPU101

CAGCAAACGAGTACCGAGAAACAGAAAGTCTACGCTGCGAAAGTCACCCATCAGGGCCTGAGCTCCCCCTACAAAGAGCTTCAACAGGGAGAGTGT 3000

- Kappa constant

Sacil

Ngomiv
Nael

TAAGCGGCCGCACTAGATAATAAGGAGATAATATGAAATATCTGCTGCCGACCGGGGGGGCTGCTGCTGCCGCCAGCCGGCGATGG
3100

Fig. 21G

optimized psi B leader

•

HC stutter

ECORV

TCGAGCTCGTCCCCCTAGAGTTCGGCGTACAGCAGCTCCGGCTCCGATATCACCGTGTCACTCCACATCAAATGAAGTAGTGCTCTAGACGCC 3300

HC shutter

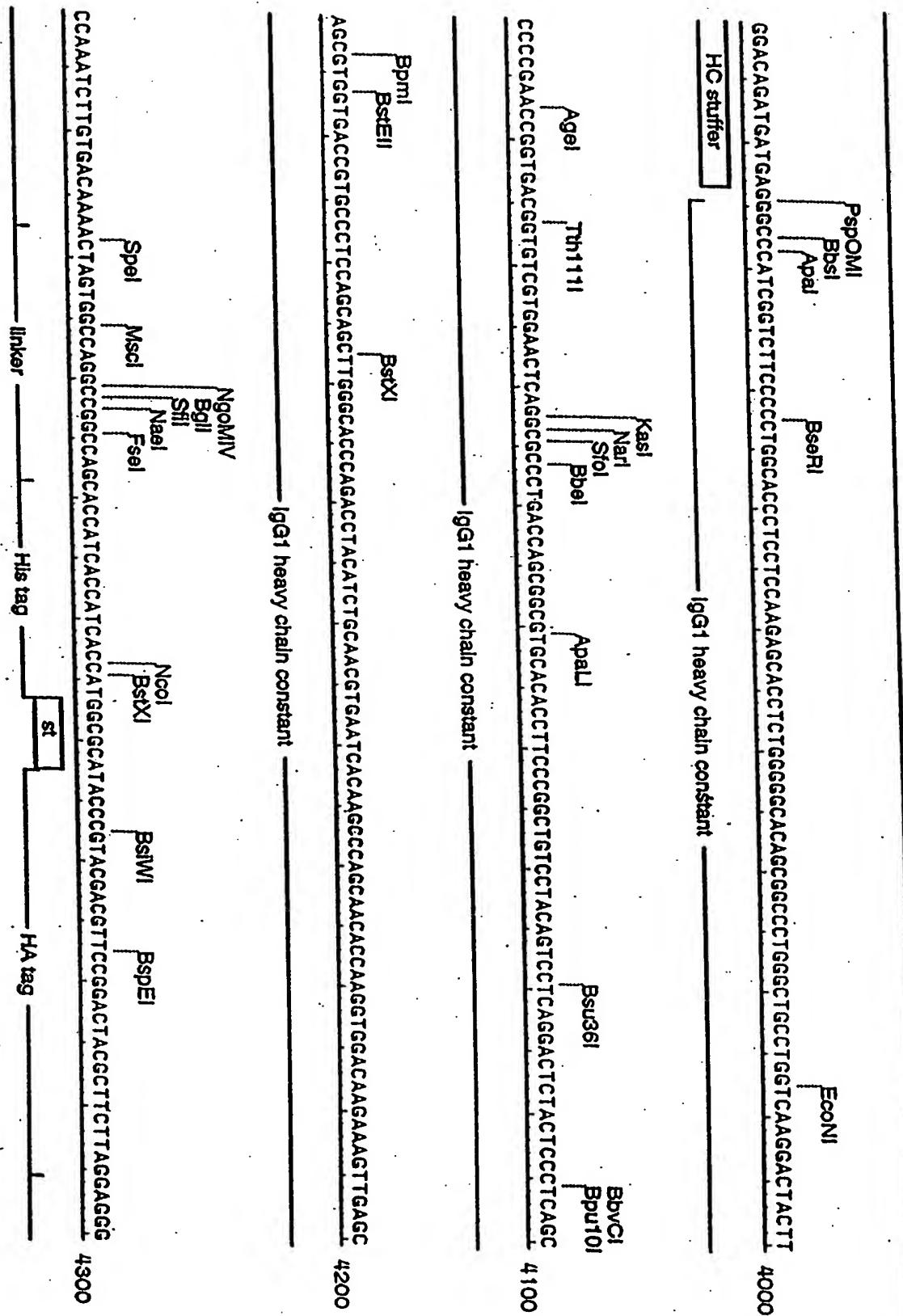


Fig. 21H

TGGGGCTCTGAGGGTGGGGCTCTGAGGGGGTCCGGCTCTGGTCCGGTGGATTATGAAAAGATGGCA 4400

AACGCTAATAAGGGGCTATGACCGAAATGCCATGAAACGCCCTACAGTCTGACGCTAAAGCAAACCTGATTCTGCGCTACTGATTACGGTCTG 4500

gene III gene III

ClaI

CTATCGATGGTTCATGGTACGGTTCCGGCCTGCTAATGGTAATGGTCTACTGGTGTGCTGGCTCTAATTCCAAATGGCTCAAAGTCGGTA 4600

gene III

XbaI BsaXI SspI BsaXI Afel NdeI
CGGTGATAATTACCTTAATGAATAATTCCGTCATATTACCTTCCTCCCTCAATCGGTTGAATGTCGCCCTTGTCTTACCCCTGGTAACCA 4700

gene III

TATGAATTTCATTGATTGACAATAACTTATCCGGGGTCTTGCCTTATATGTTGCCACCTTATGATGATTTCAGCTTG 4800

gene III

EagI NgomIV
ApaI NheI AsaI EagI NaeI FseI BglII
CTAACATACTGCGTAATAAGGAGTCTTAAGCTAGCTAATAATTAAAGCCGGCCGGCAGATCTGCTCTGAGGAGATCT 4800

gene III

Fig. 21I

Figure 22

VH: L22582 (human germline family member VH1-69)

GCAGGATTTAGGGCTGGCTCTCAGCATCCCACACTGTACAGCTGATGTGGCATCTG
TGTTTCCTTCATCGTAGATCAGGCTTGAGCTGTGAAATACCCCTGCCTCATGCATATGCA
AATAACCTGAGGTCTCTGAGATAAATATAGATATATTGGTGCCTGAGAGCATCACATAA
CAACCACATCCTCTAAAGAAGGCCCTGGGAGCACAGCTCATCACCATGGACTGGACC
TGGAGGTTCTCTTGTGGCAGCGCTACAGGTAAAGGGCTTCCTAGTCCTAAGGCTGAG
GAAGGGATCCTGGTTAGTAAAGAGGATTTATTCAACCCCTGTGTCTCTCCACAGGTGTC
CAGTCCCAGGTGCAGCTGGTGCAGTCTGGGCTGAGGTGAAGAAGCCTGGGTCTCGGTGA
AGGTCTCTGCAAGGCTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACA
GGCCCTGGACAAGGGCTGAGTGGATGGAGGGATCATCCCTATCTTGGTACAGCAAAC
TACGCACAGAAGTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCT
ACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGACAC
AGTGTGAAAACCCACATCCTGAGAGTGTCAAGAAACCTGAGGGAGAAGGCAGCTGTGCG
GGCTGAGGAGATGACAGGGTTATTAGGTTAAGGCTGTTACAAAATGGGTTATATATTG
AGAAAAAAAGAACAGTAGAAACAAGTACATACTCCTCTAATTAAAGATAATTATTCCATT
CAAGAGTCGTAATAT (SEQ ID No. 153)

JH---JH6

H3

CDR3

100

110

JH6 YYYYYGMDVWQGTTTVSS (SEQ ID No 154)

Figure 23

Vk: X12686 (human germline family member VKIII -A27)

CAGCTGCTTGCATGTCCCTCCCAGCCGCCCTGCAGTCCAGAGCCCATATCAATGCCTGG
GTCAGAGCTCTGGAGAAGAGCTGCTCAGTTAGGACCCAGAGGGAAACCATGGAAACCCAG
CGCAGCTCTCTCCTCCTGCTACTCTGGCTCCAGGTGAGGGGAACATGGGATGGTTTG
ATGTCAGTAAAACCCCTCTCAAGTCTGTTACCTGGCAACTCTGCTCAGTCAATACAATA
TAAAGCTCAATATAAAGCAATAATTCTGGCTCTTCTGGGAAGACAATGGGTTGATTAGAT
TACATGGGTGACTTTCTGTTTATTCCAATCTCAGATACCACCGGAGAAATTGTGTTGAC
GCAGTCTCCAGGCACCCCTGTCTTGTCTCCAGGGAAAGAGCCACCCCTCCTGCAGGGCCA
GTCAGAGTGTAGCAGCAGCTACTTAGCCTGGTACCGCAGAAACCTGGCCAGGCTCCAG
GTCCTCATCTATGGTGATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGT
GGGTCTGGACAGACTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTCAGTGT
ATTACTGTCAAGCAGTATGGTAGCTCACCTCCCACAGTGATTCAAGCTTGAAACAAAAACCTCT
GCAAGACCTTCATTGTTACTAGAT TATACCAGCTG (SEQ ID NO 155)

JK

L3

CDR3

--

100

JK1 WTFGQGTKVEIK (SEQ ID NO. 156)

Figure 24

pAXB116 Fab'-gVh

(SEQ ID NO 157) ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA
 (SEQ ID NO 158) M K Y L L P T A A A A G L L L L A A A Q
pelB leader
CCA GGC ATG GCG CAG GTG CAG CTG GTG CAG AGC GGC GCG GAA GTG AAA AAA CCG
P A M A Q V Q L V Q S G A E V K K P pAXB116 Fab'-gVh
(SEQ ID NO 159) Q S G A E V K K P TT-Vh(CDR3-TPO)
(SEQ ID NO 169) Q V Q L V Q S G A E V K K P L22582

CDR1-H1
GGC AGC AGC GTG AAA GTG AGC TGC AAA GCG AGC GGC GGC ACC TTT AGC AGC TAT
G S S V K V S C K A S G G T F S S Y pAXB116 Fab'-gVh
G S S V K V S C R A S G G T F N N Y TT-Vh(CDR3-TPO)
G S S V K V S C K A S G G T F S S Y L22582

GCG ATT AGC TGG GTG CGC CAG GCG CCG GGC CAG GGC CTG GAA TGG ATG GGC CAG
A I S W V R Q A P G Q G L E W M G Q pAXB116 Fab'-gVh
A I S W V R Q A P G Q G L E W M G G TT-Vh(CDR3-TPO)
A I S W V R Q A P G Q G L E W M G G L22582

CDR2-TPO
CTG ATT GAA GGC CCG ACC CTG CGC CAG TGG CTG GCG GCG CGC GCG AAC AGC CGC
L I B G P T L R Q W L A A R A N S R pAXB116 Fab'-gVh
I F P F R N T A K Y A Q H F Q G R TT-Vh(CDR3-TPO)
I I P I F G T A N Y A Q K F Q G R L22582

GTG ACC ATT ACC GCG GAT GAA AGC ACC AGC ACC GCG TAT ATG GAA CTG AGC AGC
V T I T A D E S T S T A Y M E L S S pAXB116 Fab'-gVh
V T I T A D E S T G T A Y M E L S S TT-Vh(CDR3-TPO)
V T I T A D E S T S T A Y M E L S S L22582

CTG CGC AGC GAA GAT ACC GCG GTG TAT TAT TGC GCG CGC CTG CCG ATT GAA GGC
L R S E D T A V Y Y C A R L P I E G pAXB116 Fab'-gVh
L R S E D T A I Y Y C A R L P I E G TT-Vh(CDR3-TPO)
L R S E D T A V Y Y C A R L22582

CDR3-TPO
CCG ACC CTG CGC CAG TGG CTG GCG GCG CGC GCG CCG GTG TGG GGC CAG GGC ACC
P T L R Q W L A A R A P V W G Q G T pAXB116 Fab'-gVh
P T L R Q W L A A R A P V W G Q G T TT-Vh(CDR3-TPO)

ACC GTG ACC GTG AGC AGC
 T V T V S S
 Fab'-gVh
 T V T V S A

pAXB116
 TT-Vh(CDR3-TPO)

Fig 24 Sequence of the pAXB116 Fab' Heavy chain variable region. The cDNA sequence with the best E. coli codon usage (Henaut and Danchin, 1996) and the translated amino acid sequence of pAXB116 Fab' are shown. CDR (Complementarity Determining Region) are defined by Kabat et al(1992) and the structural variability definition sequence (Chothia and Lesk, 1987) underlined and overlined, respectively. TPO peptides in heavy chain CDR2 and CDR3 of pAXB116 Fab' are indicated by double underlines and wavelines respectively. pelB leader cDNA sequences are overlined. pAXB116 Fab'-gVh denotes heavy chain variable region of human germline derived pAXB116 Fab'.

Figure 25

pAXB116 Fab' gV_k

(SEQ ID NO. 160) pelB leader ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA
 (SEQ ID NO. 161) M K Y L L P T A A A A G L L L L A A A Q

pelB leader
 CCA GCC ATG GCG GAA ATT GTG CTG ACC CAG AGC CCG GGC ACC CTG AGC CTG AGC
 P A M A E I V L T Q S P G T L S L S pAXB116 Fab'-gV_k
 (SEQ ID NO. 162) E L T Q S P G T L S L S TTV_k
 (SEQ ID NO. 170) B I V L T Q S P G T L S L S X12686

CDR1-L1
 CCG GGC GAA CGC GCG ACC CTG AGC TGC CGC GCG AGC CAG AGC GTG AGC AGC AGC
 P G E R A T L S C R A S Q S V S S S pAXB116 Fab'-gV_k
 P G E R A T L S C R A S H S V S S R A TTV_k
 P G E R A T L S C R A S Q S V S S S X12686

TAT CTG GCG TGG TAT CAG CAG AAA CCG GGC CAG GCG CCG CGC CTG CTG ATT TAT
 Y L A W Y Q Q K P G Q A P R L L I Y pAXB116 Fab'-gV_k
 Y L A W Y Q Q K P G Q A P R L L I Y TTV_k
 Y L A W Y Q Q K P G Q A P R L L I Y X12686

CDR2-L2
 GGC GCG AGC CGC GCG ACC GGC ATT CCG GAT CGC TTT AGC GGC AGC GGC AGC
 G A S S R A T G I P D R F S G S G S pAXB116 Fab'-gV_k
 G T S S R A T G I P D R F S G S G S TTV_k
 G A S S R A T G I P D R F S G S G S X12686

GGC ACC GAT TTT ACC CTG ACC ATT AGC CGC CTG GAA CCG GAA GAT TTT GCG GTG
 G T D F T L T I S R L E P E D F A V pAXB116 Fab'-gV_k
 G T D F T L T I S R L E P E D F A V TTV_k
 G T D F T L T I S R L E P E D F A V X12686

CDR3-L3
 TAT TAT TGC CAG CAG TAT GGC AGC AGC CCG TGG ACC TTT GGC CAG GGC ACC AAA
 Y Y C Q Q Y G S S P W T F G Q G T K pAXB116 Fab'-gV_k
 Y Y C Q Q Y G G S P W F G Q G T K TTV_k
 Y Y C Q Q Y G S S P X12686

G TG GAA ATT AAA
 V E I K
 Fab'-gV_k
 V E L K

pAXB116

TTV_k

Fig 25 Sequence of the pAXB116 Fab' Light chain variable region. The cDNA sequence with the best E. coli codon usage (Henaut and Danchin, 1996) and the translated amino acid sequence of pAXB116 Fab' are shown. CDR (Complementarity Determining Region) are defined by Kabat et al(1992) and the structural variability definition sequence (Chothia and Lesk, 1987) underlined and overlined, respectively. pelB leader cDNA sequences are overlined. pAXB116 Fab'-gV_k denotes light chain variable region of human germline derived pAXB116 Fab'.

Figure 26 - Primers to generate pAXB116 heavy chain

UDEC1709 : 5' primer 272 bp, containing Ncol site (5GQQ ID No. 163)

5'-CCAGGCCA/TGGCGCAGGTGCAGCTGGTGCAGAGCGGGCGGAAGTAAAAACCGGGCAGCAGCGTGAAGT
GAGCTGCAAACCGAGCGGCGGCACCTTAGCAGCTATGCGATTAGCTGGGTGCGCCAGGCGCCGGCCAGGCCCTG
GAATGGATGGGCGGATTATTCGATTTTGGCACCGGAACTATGCGCAGAAATTTCAGGGCCGCGTACCATTAC
CGCGGATGAAAGCACCGACCGCGTATATGGAACGTAGCAGCCTGCG ---'

Overlapping with UDEC1710

UDBC1710 3' primer 271 bp

5'—**GTTCCAGTCACGGTACCGGAAATAATCTTCA**CCAGGCAGCCCAGCGCCGCGGTGCCGCCG
Quiescent cell line U251

Overlapping with UDEC1711

CTGGTCTTTGCTCTCGGCCAGCGAAACACGCTCGGCTTGGTCTCGCTGCTACGGTACGGTGGT
GCCCTGGCCCCACACCGCGCGCGCGCCAGCCACTGGCGAGGGTCGGCTTCAATCGGAGGCGCGCAA
TAATACACCGCGGTATCTTCGCTCGCAGGCCTCTCAGTTCATATA—^{3'} (SEQ ID NO. 164)

Overlapping with UDEC1709

UDEC1711 3' primer (274 bp) containing XbaI site

5'—CGAGTC¹AGATTACGGGCCAGCAGTTCCGGCCGGGACGGCGGGCAGGTATGGTTTATCGCAGCT
TTCCGGTTC²CACTTTTATCCACTTTGGTGTGCTCGGTTTATGGTTCAGTTGCAAATATAGGTCGGGTGCCAGG
CTGCTGCTGGCACGGTACCAACGCTGCTCAGGCTATACAGGCCGCTGCTCTGCA³GACCCGCGGAAAGGTATGAC
GCGCTGGTCAGCGCCGCTGTTCCAGCTCACGGTCACGGTTC —3' (Seq 3) No. 165)

overlapping with UDEC1710

Figure 27 - Primers to generate pAXB116 –light chain

UDEC1712 5' primer 236 bp

5'-CCAGGCCATGGCGGAAATTGTGCTGACCCAGAGCCGGGCACCTGAGCTGAGCCGGGCGAACGGCGAC
 CCTGAGCTGCCGCGAGGCCAGAGCGTGAGCAGCTATCTGGCTGGTATCAGCAGAAACCGGGCCAGGGCGCG
 CGCCCTGATTTATGGCGCGAGCAGGCCGCGACCGGATTCGGATCGTTAGGGCAGGGCAGGGCACCG
ATTTACCGTAC-3' (SEQ ID NO. 166) Overlapping with udec1713 (24bp)

Overlapping with udec1713 (24bp)

UDEC1713 3' primer 239bp

5'-CTTCGCTTCGGCGGGATAAAAGTTGTTCAAGCAGGCACACCAACGCTCGGGTGCCTGTTTCAGTGTCTCA

overlapping with UDEC1714

TCGGTCTGGGAAAAATAAACACGGCTGGCGCCGACGGTGCCTTAATTCACTTGGTCCCTGGCCAAAGGT
 CCAOGGGTGTGCCATACTGCTGGCAATAATACACGGCAAAATCTCCGGTTOCAGGGGCTAATGGTCAGGGTAA
 AAATCGGTGCCGTG-3' (Seq ID No. 167) Overlapping with udec1712(24bp)

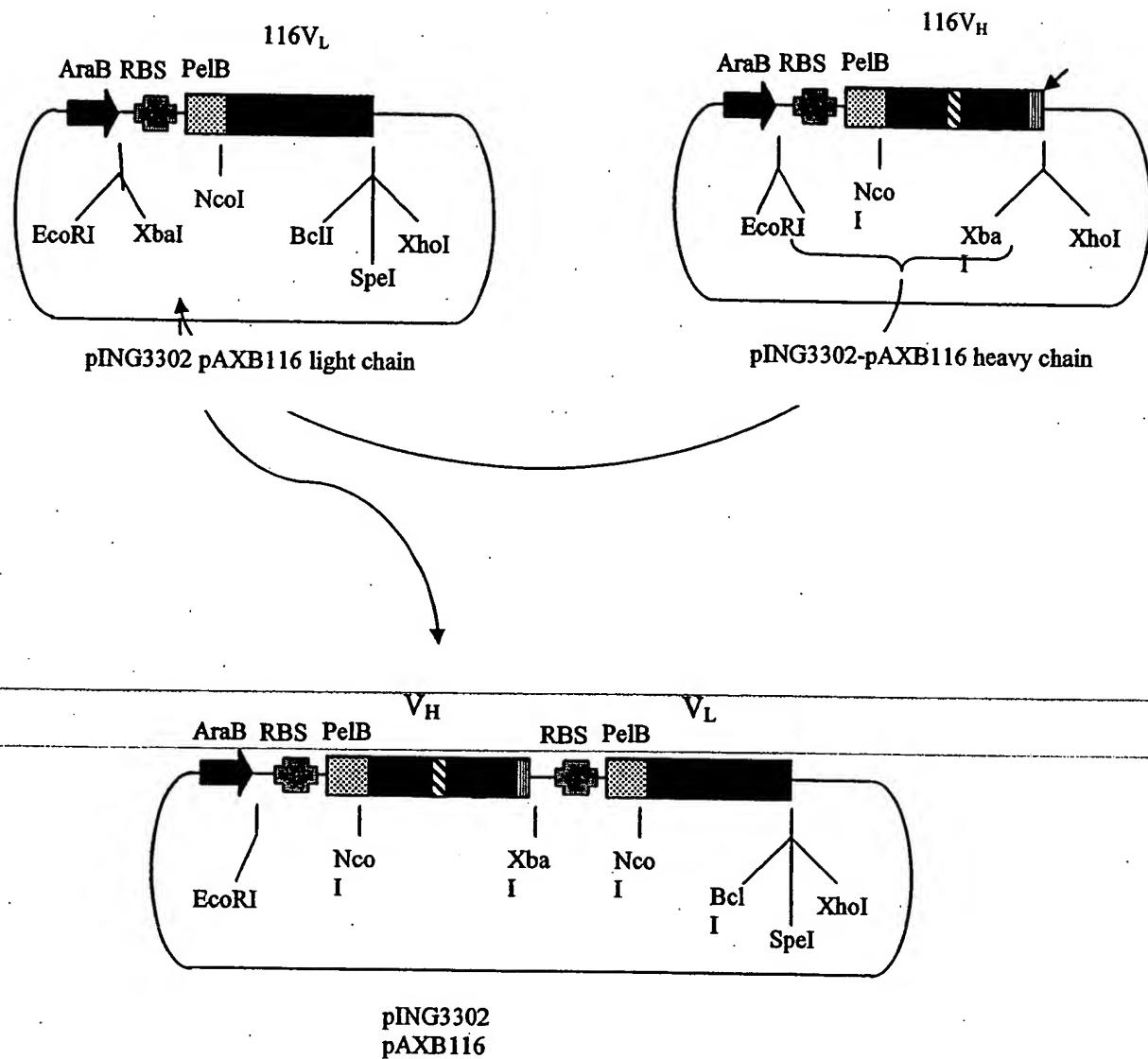
Overlapping with video1712(24hr)

UDEC1714 3' primer 245 bp

5'—GTGCTGATCATTTAGCATTCGCCGGTTAAAGCTTGGTCACCGCAGGCTCAGGCCCTGATGGGTCACCTC
GCACGCATACACTTATGTTTCTATAATCCGCTTGGTCAGGGTCAGGGTGTGCTCAGGCTATAGGTGCTATCTT
GCTATCCTGTTGGTCACGCTTCTGGCTGTGCGCTCGCAGCGGTTATCCACTTCCACTGCACTTTCGCTTCG
CGCGGATAAAAGTIG-3' (SEQ. ID no. 168) overlapping with udec1713 (26 bp)

Figure 28

Construction scheme for pING-pAXB116



116 Light Chain (SEQ. ID NO. 122):

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFLTISRLEPEDFAVYYCQQYQSSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLKADYEKHKVYA
CEVTHHQGLSLPVTKSFNRGEC.

Variable Region of 116 Light Chain (SEQ. ID NO. 123):

EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFLTISRLEPEDFAVYYCQQYQSSPWTFGQGTKVEIK

116 Heavy Chain (SEQ. ID NO. 124):

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVTITADESTSTAYMELSSLRSEDTAVYYCARLPIEGPTLRQWLAARAPVWGQGTTVTV
SSASTKGPSVFPLAPSSKSTGGTAALGCLVKDYFPEPVTVWSNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKK { **VEPKSCDKTHTCPPCP** } *APELLGGP*

end CH1 constant domain

hinge region

tail region

Variable Region of 116 Heavy Chain (SEQ. ID NO. 125):

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAIWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVTITADESTSTAYMELSSLRSEDTAVYYCARLPIEGPTLRQWLAARAPVWGQGTTVTV
SS

Clone 116. The light chain sequence is as given. The heavy chain may have several forms depending on the final antibody form. For example: the minimum sequence ending with the CH1 constant domain may result in Fab assembly but the heavy chain/light chain interaction will not be stable. Commonly, a portion of the hinge region containing a cysteine (the underlined bold portion of hinge region) may be included for a covalent interaction between the heavy and light chains. Fab'2 association would need further cysteines such as an entire IgG1 hinge region (**bold**). In this example, clone 116 was cloned in a Xoma pING3302 modified vector which includes a transition tail region (*italicized*).

Fig. 29

Figure 30

SDS-PAGE of pAXB116, culture supernatant was resolved from each other by non-reducing 4-12% SDS-PAGE (lane "Sup") and cell lysate by reducing 4-12% SDS-PAGE (lane "Lysate"). Proteins were transferred onto Hybond Nitrocellulose Sheet (Amersham) and block with TBS-0.2% Tween-20+10% (w/v) Carnation nonfat dry milk. PAXb116 were detected by HRP-conjugated goat-antiHuman (H+L)Ab (Chenicon Cat#AP112P, (Temecula, CA). The signals were detected by ECL.

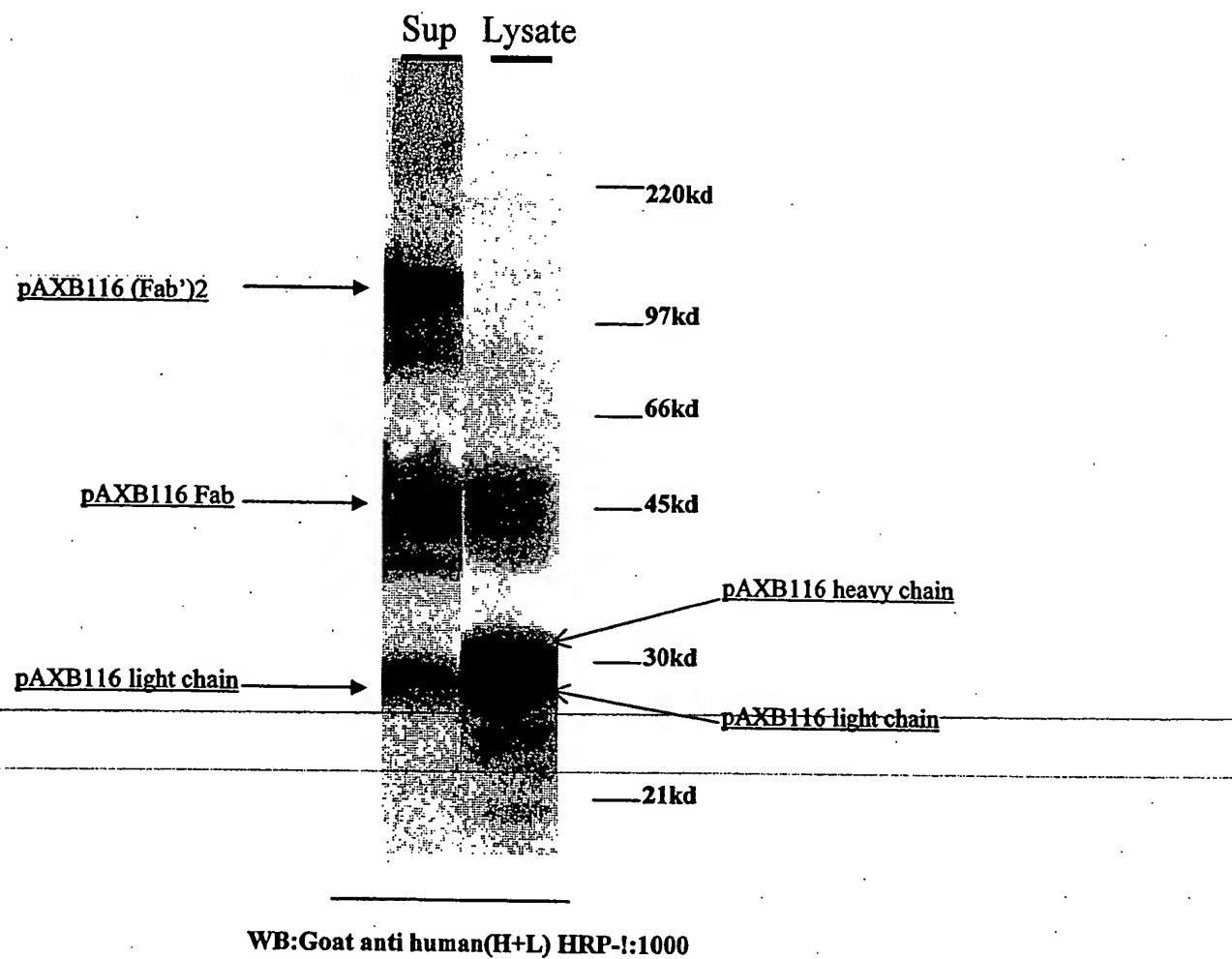


Fig. 31

Proliferative Effect of TPO on CD 34+ Cord Blood Cells

CD34⁺ cord blood cells (Poeisis) were thawed, washed, resuspended in BIT9500 serum-substituted medium (StemCell Technologies, Inc.), and plated at 3.5×10^5 per well in a 96 well flat-bottom plate with increasing concentrations of either recombinant human TPO (R&D Systems), circle, or pAXB116, square. After four days of culture at 37°C in a 5% CO₂ incubator, 1 μ Ci of ³H thymidine (Perkin Elmer) was added to each well and cells were further incubated for 16 hours. Cells were harvested with an automatic 96-well cell harvester. ³H incorporation was measured using a betaplate liquid scintillation counter (Wallac). Proliferation of cord blood cells are measured as counts per minute (CPM) and CPM values are an average of three wells.

Proliferative Effect of TPO and 116 on CD 34+ Cord Blood Cells

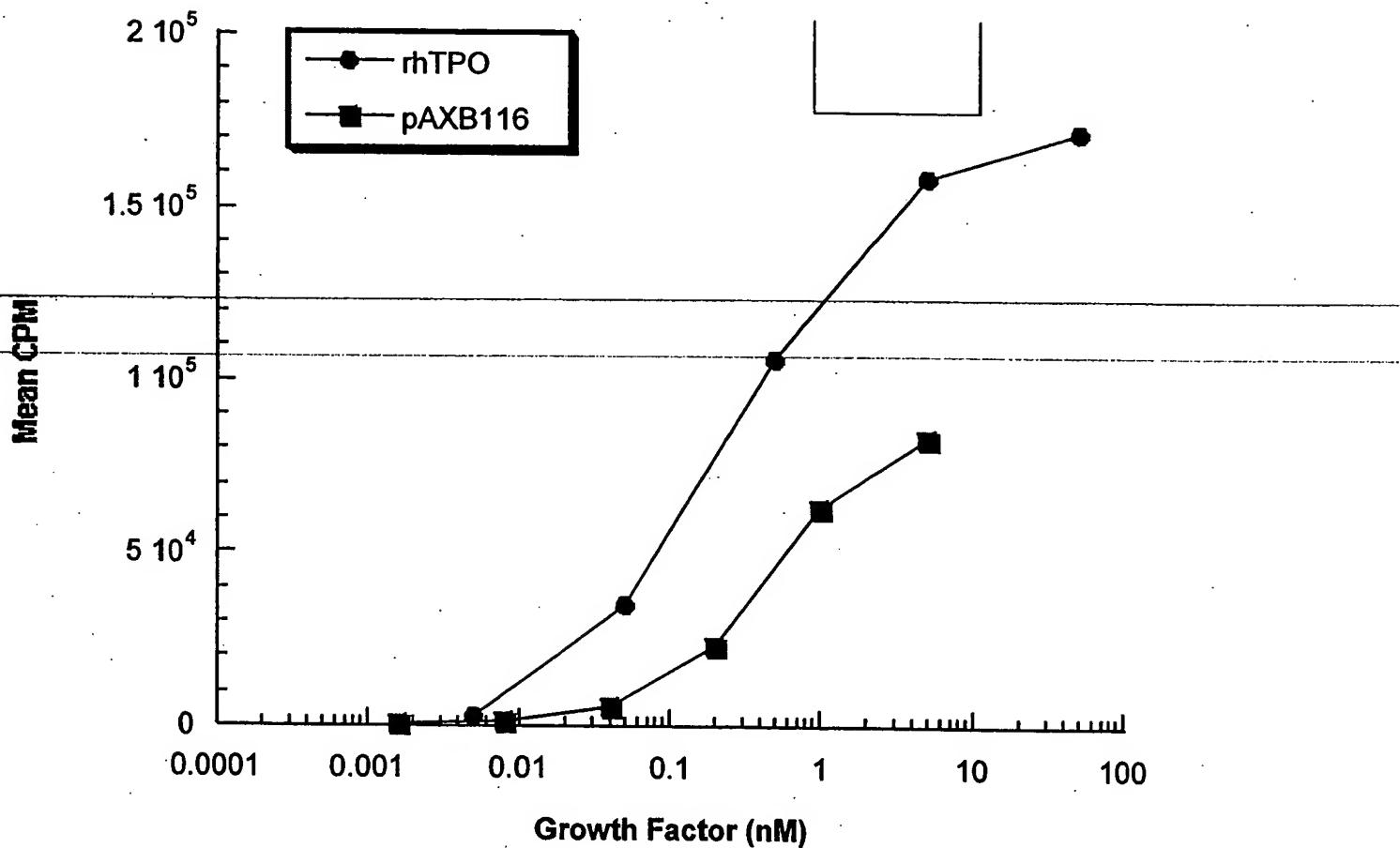


Figure 32: Activity of 116 Fab' prep #5

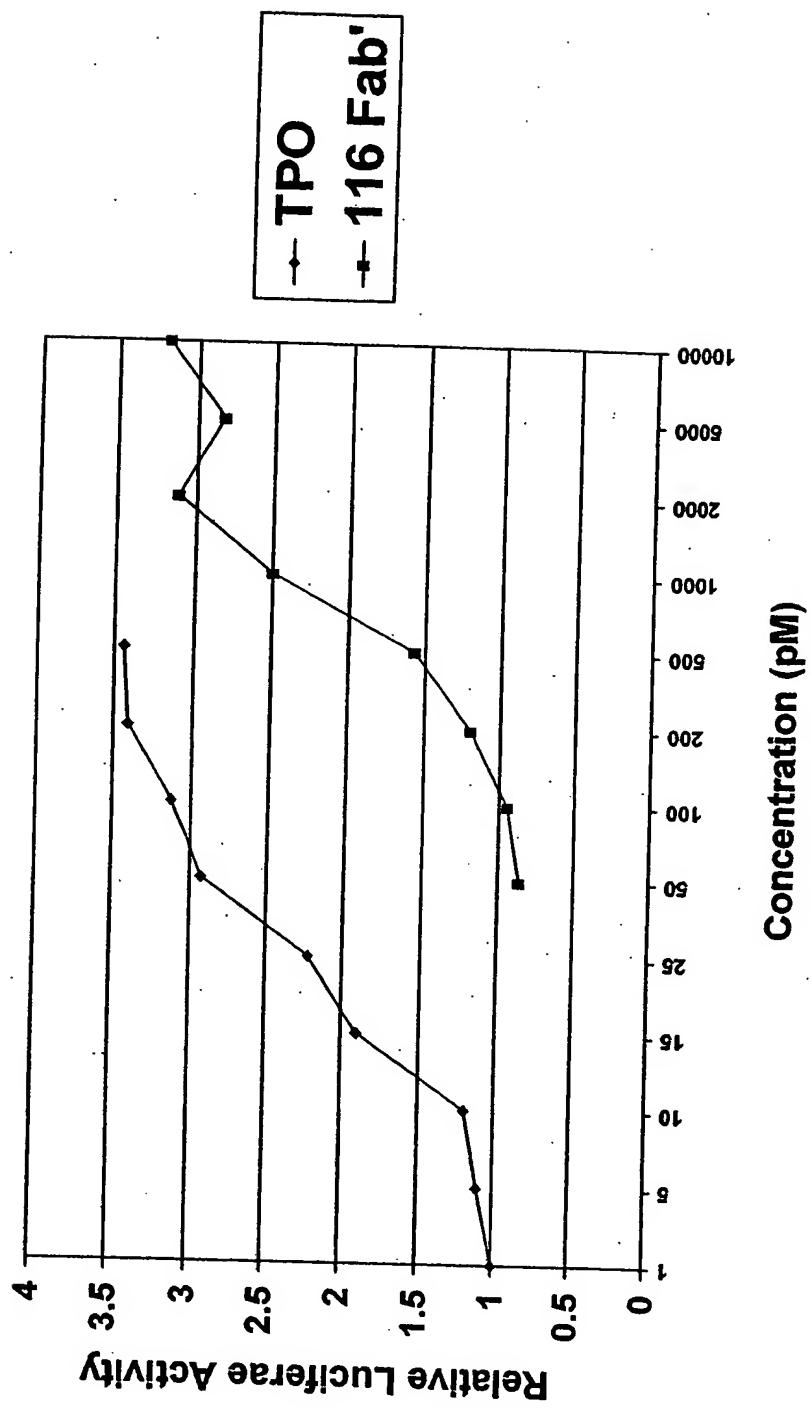


Figure 35

Sample Sequences of Heavy Chain CDR2 clones

Gly-Ile-Phe-xxx-xxx-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-xxx-xxx-Gly (SEQ. ID NO. 126)
TT backbone randomized TPO peptide randomized TT backbone

Clone

Amino Acid Sequence

- HR2-14 Gly-Ile-Phe-Ser-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly (SEQ. ID NO. 127)
- HR2-20 Gly-Ile-Phe-Pro-Gln-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-His-Gly (SEQ. ID NO. 128)
- HR2-23 Gly-Ile-Phe-Pro-Asn-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Thr-Gly-Gly (SEQ. ID NO. 129)
- HR2-28 Gly-Ile-Phe-Lys-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Gly-Gly (SEQ. ID NO. 130)
- HR2-43 Gly-Ile-Phe-Pro-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Ala-Val-Gly (SEQ. ID NO. 131)
- HR2-44 Gly-Ile-Phe-Pro-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-Leu-Gly (SEQ. ID NO. 132)
- HR2-48 Gly-Ile-Phe-Pro-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-Leu-Gly (SEQ. ID NO. 133)
- HR2-50 Gly-Ile-Phe-Pro-Tyr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-Arg-Gly (SEQ. ID NO. 134)

Figure 34 Relative Activity of 2° H2/H3-(X4b) clones

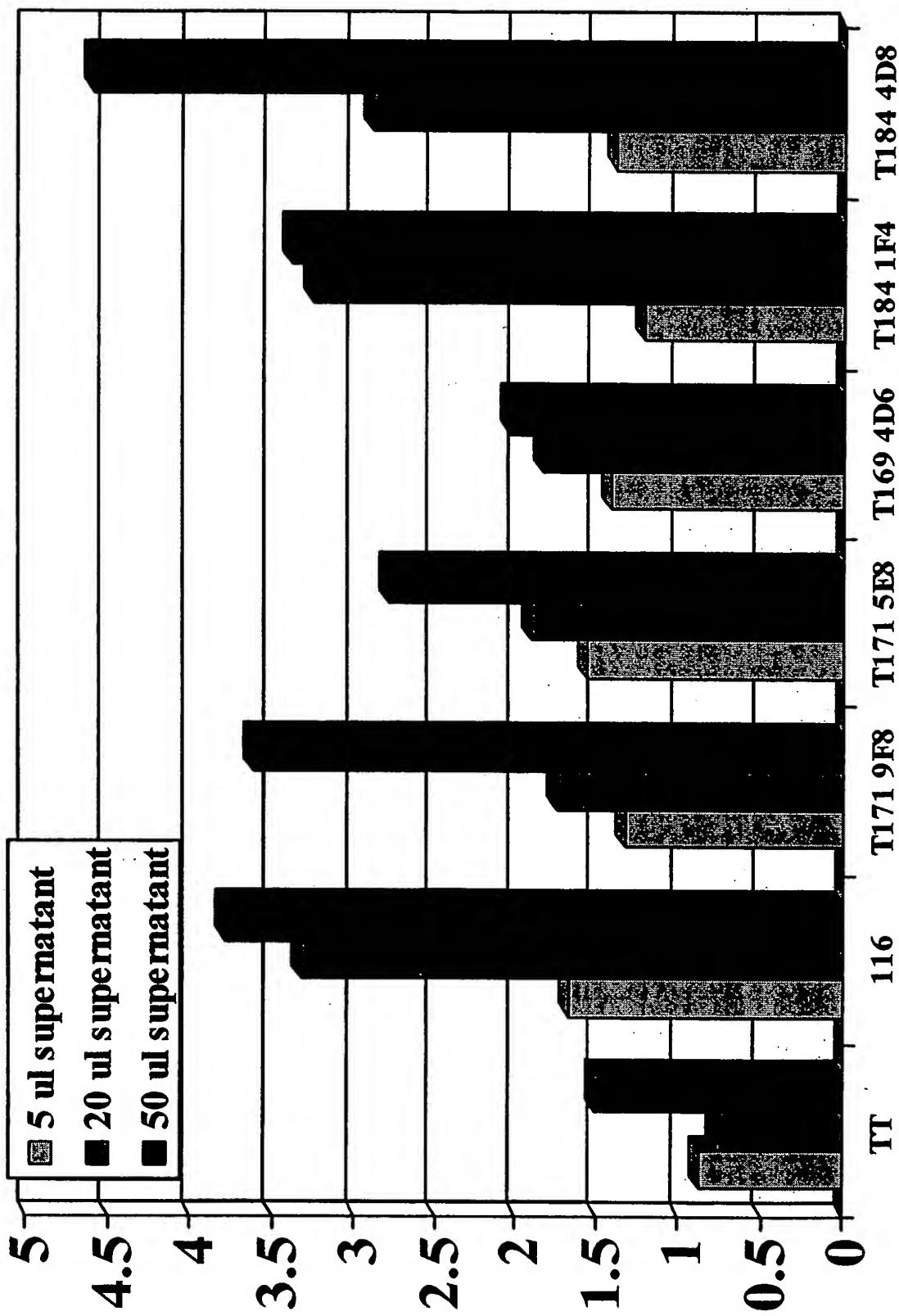


Figure 35: Optimization of the TPO placement in HC-CDR2

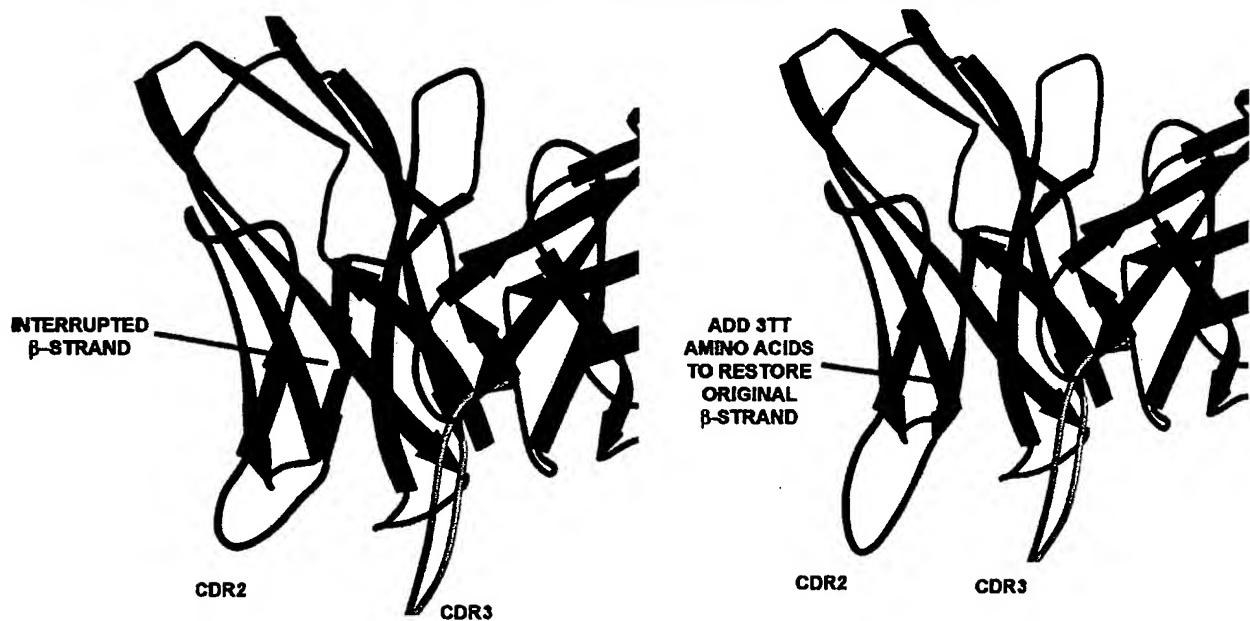


Figure 36A (seq. ID. no. 141)

Figure 36B

Pvul
 GACAACGATCGGAGGACCAAGGGAGCTAACCGCTTTTGCAACACATGGGGATCATGTAACCTGCCCTATCGTGGAAACCGAGCTGAATGAAGCC
 CTGTTGCTAGCTCCGGCTCTCGATGGCAAAAAAGTGTGTAACCCCTAGTACATTGAGCGGAACTAGCAACCCCTGGCCTCGACCTACTTGG

 BsrGI BsrDI AdII FspI
 ATACCAAAACGACGAGCTGTACACCACGATGCCGTAGCAATGGCAACAACTGGCGAAACTATTAACCTGCGAACTACTTACTCTAGCTCCGGCAAC
 TATGGTTGCTGCTGACATGTGGCTACGGACATCGTACCGTTGTTGCAACGCGTTGATAATTGACCGCTTGATGAATGAGATCGAAGGGCGTT

 AseI EdI BglII
 AATTAATAGACTGGATGGAGGCAGATAAAGTTGAGGACCACTTCTGCGCTCGGCCCTCGGCTGGCTGGTTATTGCTGATAAACTGGAGCCGGTGA
 TTAATTATCTGACCTACCTCCGCCTATTCAACGCTCTGGTGAAGACGGAGCCGGACCAAAACGACTATTAGACCTCGCCACT

 BpmI BsaI BsrDI BmrI AhdI
 GCGTGGGTCTCGCGGTATCAATTGCAAGACTGGGCCAGATGGTAAGCCCTCCGTATCGTAGTTATCTACACGACGGGAGTCAGGCAACTATGGATGAA
 CGCACCCAGAGGCCATAGTAACGTCGTGACCCGGTCTACCATCGGGAGGCGATAGCATCAATAGATGTGCTGCCCTCAGTCGTTGATACCTACTT

 DraI
 CGAAATAGACAGATCGTGAGATAGGTGCCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTACTCATATACTTTAGATTGATTAAACCTTC
 GCTTTATCTGCTAGCGACTATCCACGGAGTACTAATTGTAACCATTGACAGTCTGGTCAAAATGAGTATATGAAATCTAACTTGTGAAG

 DraI BspHII
 ATTTTAATTAAAAGGATCTAGGTGAAGATCCTTTTGATAATCTGATGACCAAAATCCCTAACGTGAGTTTCTGGTCAACTGAGGGTCAGAGCCCGT
 TAAAGATTAATTCTCTAGATCCACTCTAGGAAAAACTATTAGAGTACTGGTTAGGGAAATTGCACTCAAAAGCAAGGTGACTCGCAGTCGGGCA

 AGAAAAGATCAAAGGATCTCTGAGATCTTCTGCGCTAACCTGCTGCTTGCAACAAAAACCCGCTAGCAGCGTGGTTGTTGCC
 TCTTTCTAGTTCTAGAAGAACTCTAGGAAAAAGAGCGCATTAGACGACGAACTGGTTTGTGTTTGTGGCAGTGGTCAACAAACAGGCG

 Eco57I
 GATCAAGAGCTACCAACTCTTCTCGAAGGTAACCTGGCTCAGCAGAGCGAGATACCAAATACTGTCCTCTAGTGTAGCCGTAGTTAGCCACACT
 CTAGTTCTCGATGGTGAGAAAAAGGCTTCATTGACCGAAGTCGTCCTGGCTCTGGTTATGACAGGAAGATCACATCGCATCAATCCGGTGGTGA

 AAVNI
 TCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGTAATCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCGTTCTTACCGGGTTGGACTC
 AGTTCTTGAGACATCGTGGCGATGATGGAGCGAGACGATTAGGACAATGGTCACCGACGACGGTACCGCTATTCAAGCACAGAATGGCCAACCTGAG

 ApaI
 AAGACGATAGTTACCGATAAGGCGCAGCGGTGGCTGAAACGGGGGTTCTGCAACACGCCAGCTTGAGCGAACGACCTACACCGAACCTGAGATAAC
 TTCTGCTATCAATGGCTTATCCCGCTGCCAGCCGACTTGGCCCCCAAGCACGTTGTCGGGTCGAACCTCGCTTCTGGATGTTGCTTGACTCTATG
 100 120 130 140 150 160 170 180 190 200

Figure 36C

CTACRGCGTGRGCTATGAGRAAGCGCCACGCTTCCGAGGGAGRAAGGCGRACGGTATCCGGTAAAGCGCAGGGTCGGACAGGGAGGCACGCGACGAGGG
GATGTCGCACTCGATRCCTTTCCGGTTCGAGGGCTTCCCTCTTCCGCTGTCCATRGCCATTCGCCCTCCAGGCTT>CTCTCAGCGTGTCCCC
AGCTTCCAGGGGAAACGCCCTGGTATCTTTRAGTCCCTGCGGGTTTCGCCACCTCTGACTTGAGCTCGATTGTTGTCGCTCGTCAAGGGGGCGGRG
TCGAGGGTCCCCCTTGCGAACATAGAAATACTCAGGACAGCCAAAGCGGTGGAGACTGAACTCGCAGCTAAACACATACGAGCAGTCCCCCGCTC
CCATGGRAAACGCCACGGCTTTTACGGTTCCCTGGCCTTTGCTGGCCTTTGCTACATGTTCTCTGCGTTATCCCCCTGATTCTGTC
GGATACCTTTTGCGGTGTTGCGCCGGAAATGCCAAGGACCGGAAACGAGTGTRACAGGAAAGGACGCAATAGGGGACTAGAAC
GAATACCGTATTAACGCCCTTGAGTGRGCCTGAGTACCGCTCGCCGCRGCCGACCGAGCGCAGGTCAGTGGCGAGGGAGCGGAAAGGCGCCCA
CTATTGGCATTAATGGCGGAAACTCACTCGACTATGGCAGGCGCTGGCTTGCTCGCTGCTAGTCAGTCAGTCCTTGCGCTTCTGCGGGTT
TRACGCAACGCCCTCCTCCCGCGCTGGCCGATTCAATTAGCAGCTGGCAAGCAGGTTCCGACTGGAAAGCGGGCAGTGAGCGCAGCAGCAATTAA
ATCCGTTGGCGAGAGGGGGCGCAGACCCGGCTAGTATTACGTCGACCGTGTCCAGGCTGACCTTTCGCCCGTCAGCGTGTGGTT
TGAGGTTAGCTCACTCATTRGGCACCCAGGCTTCACTTTATGCTTCCGGCTGTTATGTTGTTGGGATTTGAGGGAGTRACATTGAAATTGAGA
ACACTCAATCGAGTGAATCCGTTGGGTCGAGATGAAATACGAAAGGCCAGCATACACACCTTACACTCGCTTATTGTTACCTTAAAGTC
BsaXI' AseI PvuII BstBI MspI EcoRI
TACGCAACGCCCTCCTCCCGCGCTGGCCGATTCAATTAGCAGCTGGCAAGCAGGTTCCGACTGGAAAGCGGGCAGTGAGCGCAGCAGCAATTAA
ATCCGTTGGCGAGAGGGGGCGCAGACCCGGCTAGTATTACGTCGACCGTGTCCAGGCTGACCTTTCGCCCGTCAGCGTGTGGTT
TGAGGTTAGCTCACTCATTRGGCACCCAGGCTTCACTTTATGCTTCCGGCTGTTATGTTGTTGGGATTTGAGGGAGTRACATTGAAATTGAGA
ACACTCAATCGAGTGAATCCGTTGGGTCGAGATGAAATACGAAAGGCCAGCATACACACCTTACACTCGCTTATTGTTACCTTAAAGTC
BsaXI' NruI BstI BglII SfiI EcoICRI SacI
GGATTTAAATGAAAGGAGCAGCTATCGCGATTGCACTGGCACTGGCTGGCTGGCTACCGTGACCGACCCGGGTCGAGCTGAAATTGTCGAGG
CTTAAATTTACTTTCTGTCGATGCGCTACGTCACCGTGACCGACCCGGGTCGAGCTGAGCTTACACGAGCTGAGCTTACACGAGCTGGCT
D N Q I N P K K T R I A I R V R L R G F A T V A Q R R E L E I V L T O
omtP leader VL 116
XbaI SmaI SstI Bpu10I XbaI SmaI SstI Bpu10I Bpu10I BglII SfiI EcoICRI SacI
AGCCGGGCCAGCCCTGAGCTTGAGCCGGCGAACCGCGACCCCTGAGCTGCCCGCGAGGCCAGAGCGTGAGCGAGCTATCTTGCGTGGTATCAGCGAG
TGGGGCCCGTGGGACTCGGACTCGGGCCGCTTGCGCTGGGACTCGAGCGCAGCTGGCTCGAGCTGAGCTTACACGAGCTGAGCTTACACGAGCTGGCT
S P G T L S L S P G E R R T E S C R A S Q S V S S S Y L A V Y Q Q
VL 116
KasI NciI SstI BbsI BsmI BspEI
AACCGGGCCAGGCCGCGCCCTGCTGAGTTTATGGCGCAAGCAGCGCGACCCGGCATCCGGATTCGGCTTGCGGAGCGGGACCGATTTTAC
TGGGGCCCGTCCCGGGCGAGCTGAGCTTACCGCGCTCGTGGCGCCTGGCGTAAAGGCTAGCGAAATCGCACTCGCCGTGCGCTGGCTTACACGAGCTGGCT
K P G Q R P R E L L I Y G A S S R A T G I P D R F S G S Q S G T D F T
VL 116
XbaI BstXI MscI
CTACGCGTGRGCTATGAGRAAGCGCCACGCTTCCGAGGGAGRAAGGCGRACGGTATCCGGTAAAGCGCAGGGTCGGACAGGGCACCAAGTGGAA
GGACTGGTAAATCGGCGGAGCTTGGCTTCTTACACGCCCATTAACGGCTGCTACCGCTGCTACCGCTGCTGGGACCCCTGGGAAACCGGTCGGTGGTTTACCTT
L T I S R L E P E D F R V Y Y C Q Q Y G S S P V T F G Q G T K V E
VL 116

Figure 50D

Figure 36E

Bpu10I BstEII

TATATAGCCTGAGCAGCGTGGTACCGTGCAGAGCAGCTGGGACCCAGACCTATTTGCAACGTGAAACATAACCGAGCAACACCAAAGTGGAA 41C
ATATATCGGAACTCGTGGCAGCACTGGCAGGCTGGTCTGGATATAACGTTGCACTTGGTATTTGGCTCGTTGGTTAACCT

L Y S L S S V V T Y P S S L G T Q T Y I C N V N H K P S N T K V D
codon optimized CH1

NgoMIV
BglI
SmaI
SphI
MscI
Nael
FseI

TAAAAAAGTGGAAACCGAAAAGCTGGATAAAACTAGTGGCAGGGCGGCAGCACCATCACCATGGCGCATACCGTACGACGTTCGGACTAC 42C
ATTTTTTCACTTGCTTTTCGACGCTATTTGATEACGGTCCGGCCTGGTCGTGGTAGTGGTAGTGGTACCCGCTGGCATGCTGCAAGGCCTGATG

K K Y E P K S C D K T S G Q A G Q H H H H H H H G A Y P Y D V P D Y
codon optimized CH1

Linker His 6 tag HA tag

GCTCTTAGGAGGGTGGTGGCTCTGGGGTGGCGGTTCTAGGGTGGCGCTCTGAGGGAGGGCGGTTCDGGTGGCTCTGGTCCGGTGAATTGGATT 43C
CGAAGAACCTCCACCAACCCGAGACTCCCACCGCCAAAGACTCCACCGCCGAGACTCCCTCCGCAAGGCACCCACCGAGACCAAGGCCACTAAACCTAA
(seq ID no. 171)

A S E G G G S E G G G S E G G G S G G G S G S G D F D
gene III fragment

ATGAAAAGATGGCAACGCTAATAAGGGGCTATGACGGAAAATGCCGATGAAAAGCGCCTACAGTCTGACGCTAAAGGAAACTGATTCTGTCGCTAC 44C
TACTTTCTACCGTTGGATTATTCACCGATACTGGCTTACGCTACTTTGGGATGTCAGACTGGGATTTCGATGAACTAAGCACAGCAG

Y E K H A N A N K G A M T E N A D E N A L O S D A K G K L O S V A T
gene III fragment

ClaI

TGATTACGGTGCTGCTATCGATGGTTGATTGGTGACGTTCCGGCTCTCTAATGGTAATGGCTACTGGTGATTGGCTCTAATTCCAAATG 45C
ACTAATGCCACGACGATAGCTACCAAAAGTAACCACTGCAAAGGCCGAAACGATTACCAATTACACGATGACCACTAAACGCCGAGATTAGGGTTAC

D Y G A A I D G F I G D Y S G L A N G N G A T G D F A G S N S Q H
gene III fragment

XbaI BsaXI SspI BsaXI

GCTCAAGTCGGTGACGGTGAATAATTACCTTTAATGAATAATTCCGGTCAATATTACCTTCCCTCCCTAAATGGTTGAATGTCGGCCCTTTGCTTTA 46C
CGAGTTCAACTGCCACTATTAAGTGGAAATTACTTAAAGGCAAGTTAAATGAAAGGGAGGGAGTTAGCCAACCTACAGGGAAACAGAAAT

A Q V G D S D N S P L M N N F R Q Y L P S L P Q S V E C R P F V F
gene III fragment

NdeI NdeI

GGCTGGTAAACCATATGAATTTCGATTTGATTTGATGACAAAATAAACCTATTCCGGTGGTGTTGGTTTTGTTATATGTTGCCACCTTATGTTATG 47C
CGCGACCATTTGGTATACTTAAAGATAACACTGTTTATTGAATAAGGCAACAGAACGAAAGAAAATACAAACGGTGGAAATACATA

S A G K P Y E F S I D C D K I N L F R G V F A F L L Y V A T F M Y V
gene III fragment

AluI NheI AseI Nofl BglII

ATTTCTACGGTTGCTAACATACTGCGTAATAAGGAGTCTTAAGCTAGCTAACCTAAATTAAAGCGGCCGCAAGATCTGCTCTGAGGAGGATCT 4783
TAAAAGATGCAAACGATTTGATGACGCATTACCTCAGAATTGATCGATGATTAATTAAATTGCGCCGGCTGAGCAGAGACTCCTCTAGA

F S T F A N I L R N K E S
gene III fragment

Figure 37 Relative Activity of 116 Mutants

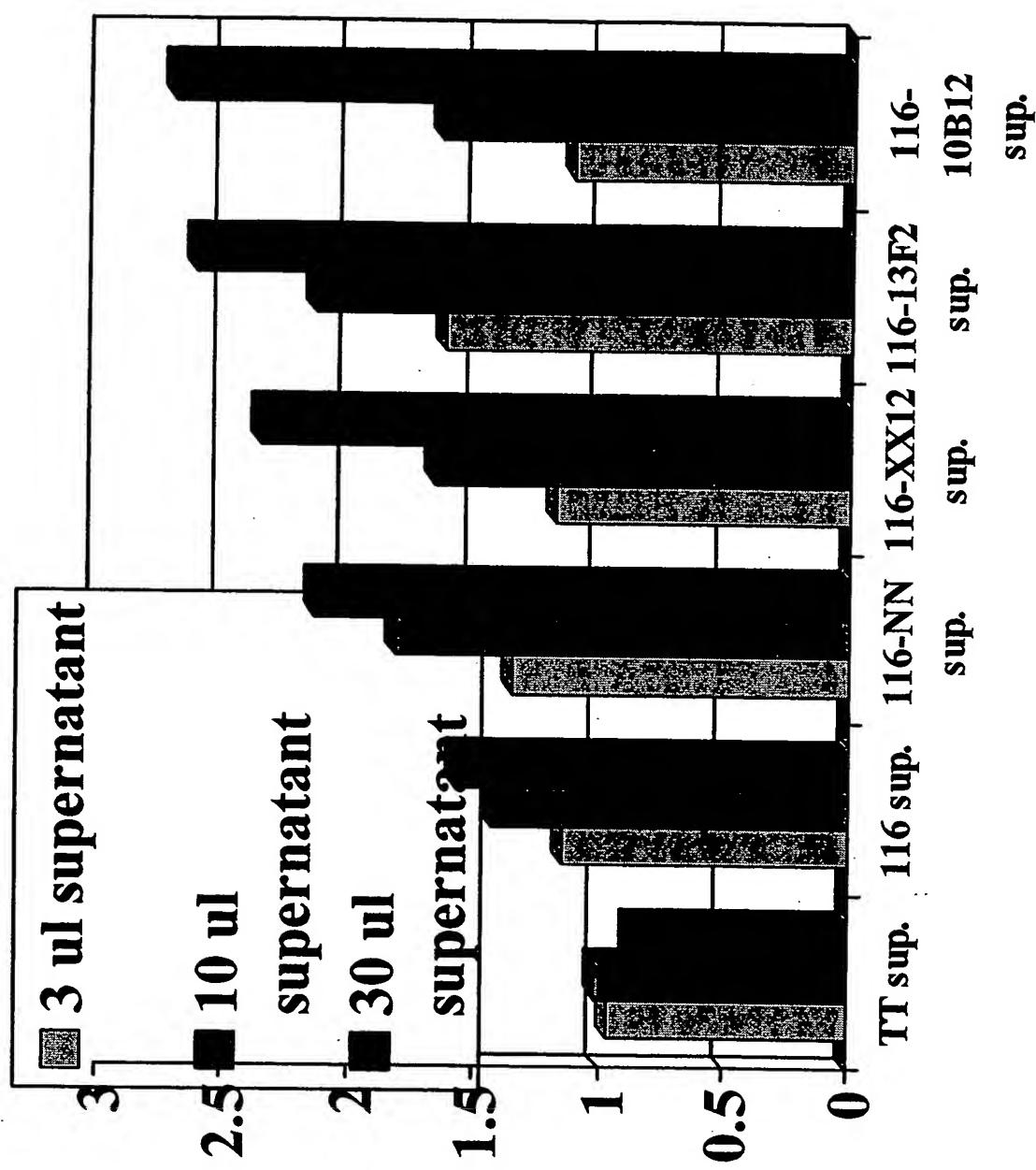


Figure 38 116 Variants Alignment

	10	20	30	
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S			pRL5-116 (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F N			pRL5-116 NN (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F G			pRL5-116 10B12 (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F Q			pRL5-116 13F2 (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F P			pRL5-116 XX12 (VH)

	40	50	60	
31	S Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 (VH)
31	N Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 NN (VH)
31	E Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 10B12 (VH)
31	D Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 13F2 (VH)
31	R Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W			pRL5-116 XX12 (VH)

	70	80	90	
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 NN (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 10B12 (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 13F2 (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E			pRL5-116 XX12 (VH)

	100	110	120	
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 NN (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 10B12 (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 13F2 (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q			pRL5-116 XX12 (VH)

	121	121	121	121	121	
121	G T T V T V S S (SEQ. ID No. 147)					pRL5-116 (VH)
121	G T T V T V S S (SEQ. ID No. 148)					pRL5-116 NN (VH)
121	G T T V T V S S (SEQ. ID No. 149)					pRL5-116 10B12 (VH)
121	G T T V T V S S (SEQ. ID No. 150)					pRL5-116 13F2 (VH)
121	G T T V T V S S (SEQ. ID No. 151)					pRL5-116 XX12 (VH)